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(71) Applicant *(for all designated States except US)*: **ORIDIGM CORPORATION** [US/US]; Suite 200, 4010 Stone Way North, Seattle, WA 98103 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BURNS, Mark, Robert** [US/US]; 226 NW 184th Street, Shoreline, WA 98177 (US). **GRAMINSKI, Gerard, F.** [US/US]; 15733 Palatine Avenue N, Shoreline, WA 98133 (US). **BANDUIR, Nand** [IN/US]; 313 Quail Ridge Drive, Plainsboro, NJ 08536 (US).

(74) Agent: **LAU, Kawai**; Morrison & Foerster LLP, Suite 500, 3811 Valley Centre Drive, San Diego, CA 92130-2332 (US).

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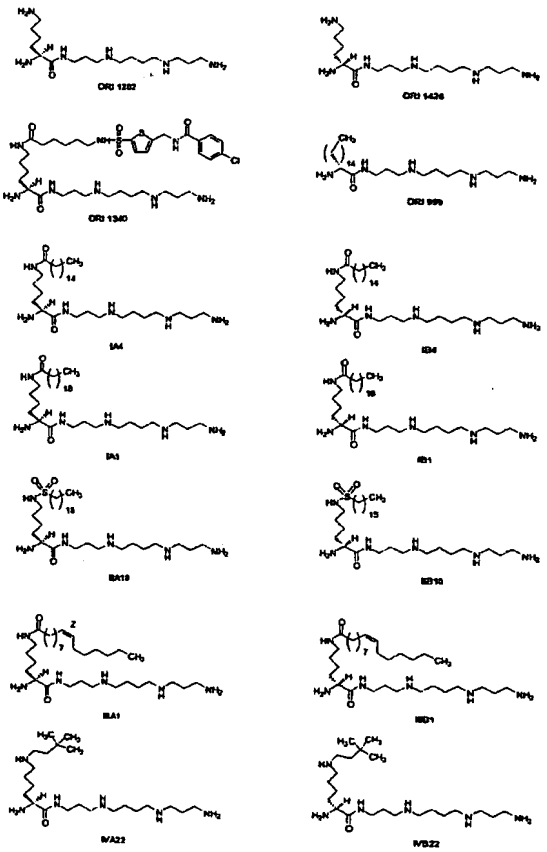

(57) Abstract: The disclosed invention provides new polyamine analogs and derivatives containing a hydrophobic region and a polyamine region as well as methods and compositions for their use.

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Hydrophobic Polyamine Analogs and Methods for their Use

FIELD OF THE INVENTION

The invention in the field of chemistry and biochemistry relates to the synthesis and use of a novel class of polyamine transport inhibitor compounds. These compounds have pharmacological and/or agricultural applications as well as uses in analytical and preparative assays relating to polyamine transport. As pharmaceuticals, these compounds are used to treat disorders of undesired cell proliferation, especially in eukaryotic cells, alone or in combination with other agents such as polyamine synthesis inhibitors.

BACKGROUND OF THE INVENTION

Decades of research on the myriad of biological activities that the polyamines, putrescine, spermidine and spermine play in cellular processes have shown the profound role they play in life (Cohen, S.S., "A Guide to the Polyamines" **1998,** Oxford University Press, New York). As polycations at physiological pH, they bind tightly to and strongly modulate the biological activities of all of the anionic cellular components.

Many stimuli involved in both normal and neoplastic growth activate the polyamine biosynthetic pathway. A great number of multidisciplinary studies have shown that the intracellular concentrations of the polyamines is highly regulated at many steps in their biosynthesis, catabolism and transport. The fact that cells contain such complex apparatus for the tight control of the levels of these molecules shows that only a very narrow concentration range is tolerated.

Polyamine transport into mammalian cells is energy and temperature dependent, saturable, carrier mediated and operates against a substantial concentration gradient (Seiler, N. et al. Polyamine transport in mammalian cells. *Int. J. Biochem.* **1990,** *22,* 211-218; Khan, N.A.; Quemener, V. et al. Characterization of polyamine transport pathways, in *Neuropharmacology of Polyamines* (Carter, C., ed.), **1994,** Academic, San Diego, pp. 37-60). Ample experimental proof exists that polyamine concentration homeostasis is mediated via this transport system. Changes in the requirements for polyamines in response to growth stimulation is reflected by increases in the transport activity. Stimulation of human fibroblasts to cell proliferation by serum or epidermal growth factor was followed by an 18-100 fold increase in the uptake of putrescine (DiPasquale, A. et al.

Epidermal growth factor stimulates putrescine transport and ornithine decarboxylase activity in cultures human fibroblasts. *Exp. Cell Res.* **1978**, *116*, 317-323; Pohjanpelto, P. Putrescine transport is greatly increased in human fibroblasts initiated to proliferate. *J. Cell Biol.* **1976**, *68*, 512-520). Tumors have been shown to have an increased rate of putrescine uptake (Volkow, N. et al. Labeled putrescine as a probe in brain tumors. *Science*, **1983**, *221*, 673-675; Moulinoux, J-P. et al. Biological significance of circulating polyamines in oncology. *Cell. Mol. Biol.* **1991**, *37*, 773-783).

Inhibition of polyamine biosynthesis in cells in culture by α-difluoromethylornithine (DFMO), a well-studied mechanism-based inhibitor of ODC, causes a substantial depletion of intracellular putrescine and spermidine with resultant cell growth inhibition. Upon supplementing the culture media with exogenous polyamines this depletion causes transport activity to rise several-fold (Bogle, R.G. et al. Endothelial polyamine uptake: selective stimulation by L-arginine deprivation or polyamine depletion. *Am. J. Physiol.* **1994**, *266*, C776-C783; Alhonen-Hongisto, L. et al. Intracellular putrescine deprivation induces uptake of the natural polyamines and methylglyoxal bis(guanylhydrazone). *Biochem. J.* **1980**, *192*, 941-945). The cells then returned to their original rate of growth.

Genes for the polyamine transport protein or complex have been cloned from *Escherichia coli* and yeast (Kashiwagi, K. et al. *J. Biol. Chem.* **1990**, *265*, 20893-20897; Tomitori, H. et al. Identification of a gene for a polyamine transport protein in yeast. *J. Biol. Chem.* **1999**, *274*, 3265-3267). The genes for the mammalian transporter await identification. A subunit of the transporter from *E. coli* has been crystallized and its X-ray structure has been determined (Sugiyama, S. et al. Crystal structure of PotD, the primary receptor of the polyamine transport system in Escherichia Coli. *J. Biol. Chem.* **1996**, *271*, 9519-9525). This structure represents one of a few but growing number solved for spermidine-binding proteins. Since this structure was determined on a prokaryotic species its use in the design of mammalian transport inhibitors was deemed to be of limited value.

Several researchers have studied the ability of polyamine analogs to inhibit the uptake of ^{3}H-spermidine into cells. Bergeron and coworkers studied the effect of addition of different alkyl group substitutions on the terminal nitrogen atoms of spermidine or spermine analogs (Bergeron, R.J. et al. Antiproliferative properties of polyamine analogs: a structure-activity study. *J. Med. Chem.* **1994**, *37*, 3464-3476). They showed that larger alkyl groups diminished the ability to prevent uptake of radiolabeled spermidine. They

later concluded that increases in the number of methylenes between the nitrogen atoms decreased the ability to compete for ^{3}H spermidine uptake (Bergeron, R.J. et al. A comparison of structure-activity relationships between spermidine and spermine antineoplastics. *J. Med. Chem.* **1997**, *40*, 1475-1494). They also concluded that the polyamine transport apparatus requires only three cationic centers for polyamine recognition and transport (Porter, C.W. et al. *J. Cancer Res.* **1984**, *44*, 126-128). Two groups have analyzed literature examples of the polyamine analogs' ability to inhibit ^{3}H spermidine uptake into L1210 cells by CoMFA and QSAR methods (Li, Y. et al. Comparative molecular field analysis-based predictive model of structure-function relationships of polyamine transport inhibitors in L1210 cells. *Cancer Res.* **1997**, *57*, 234-239; Xia, C.Q. et al. QSAR analysis of polyamine transport inhibitors in L1210 cells. *J. Drug Target.* **1998**, *6*, 65-77).

A radiochemical assay is used for biochemical analysis of transport and has been used to study polyamine transport in yeast and a variety of mammalian cells (Kakinuma, Y. *et al., Biochem. Biophys. Res. Comm.* 216:985-992, 1995; Seiler, N. *et al., Int. J. Biochem. Cell Biol.* 28:843-861, 1996). See, for example Huber, M. *et al. Cancer Res.* 55:934-943, 1995.

WO 99/03823 and its corresponding U.S. Patent Application Serial No. 09/341,400, filed July 6, 1999, (both of which are hereby incorporated in their entireties as if fully set forth) as well as the recent publications of Burns, M.R.; Carlson, C.L.; Vanderwerf, S.M.; Ziemer, J.R.; Weeks, R.S.; Cai, F.; Webb, H.K.; Graminski, G.F. Amino acid/spermine conjugates: polyamine amides as potent spermidine uptake inhibitors. *J. Med. Chem.* **2001**, *44*, 3632-44 and Graminski, G.F.; Carlson, C.L.; Ziemer, J.R.; Cai, F., Vermeulen, N.M.; Vanderwerf, S.M.; Burns, M.R. Synthesis of bis-spermine dimers that are potent polyamine transport inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 35-40 describe some extremely potent polyamine transport inhibitors.

Citation of any reference herein is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these documents.

DISCLOSURE OF THE INVENTION

The present invention is directed to novel polyamine analogs and derivatives and methods for their use as drugs, as agricultural or as environmentally useful agents. These novel polyamine analogs and derivatives comprise a hydrophobic moiety covalently attached to a polyamine moiety. These novel PA analogs can be considered to have amphipathic character (hydrophobic as well as charged portions). The polyamine analogs and derivatives of the invention include those that may be viewed as a polyamine acylated with a hydrophobic acyl group, where acylation is by formation of either an amide or a sulfonamide linkage. While the linkage between the hydrophobic acyl group and the polyamine moiety may occur at any amine group within the polyamine, linkages to a primary amine functionality are preferred.

The analogs and derivatives of the invention are potent inhibitors of cellular polyamine transport. Without being bound by theory, they are inferred to bind to a cell's polyamine transporter apparatus with very high affinity. They may be used independently or in combination with the inhibition of cellular polyamine synthesis, even in the presence of exogenously supplied spermidine, to inhibit cell growth and proliferation.

The analogs and derivatives of the invention include those encompassed by the following formula I:

R-X-polyamine

wherein R is selected from H or from the group of a straight or branched C1-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy; a C1-8 alicyclic; a single or multiring aryl substituted aliphatic; an aliphatic-substituted single or multiring aromatic; a single or multiring heterocyclic; a single or multiring heterocyclic aliphatic; a C1-10 alkyl; an aryl sulfonyl; or cyano;

"X" may be -CO-, -SO_2-, or -CH_2-, and

"polyamine" may be any naturally occurring, such as putrescine, spermine or spermidine, or synthetically produced polyamine.

Preferably, R is at least about C5, at least about C10, at least about C11, at least about C12, at least about C13, at least about C14, at least about C15, at least about C16, at least about C17, at least about C18, at least about C19, at least about C20, or at least about C22.

The linkage between X and the polyamine may be direct, wherein there are no atoms between X and the nitrogen of the amine group of the polyamine, or indirect, where there may be one or more atoms between X and the nitrogen of the amine group of the polyamine. The linkage between X and the polyamine may occur via any amino group within the polyamine, although a primary amino group is used in preferred embodiments of the invention.

In preferred embodiments of the invention where the linkage between X and the polyamine is indirect, the intervening one or more atoms are preferably those of an amino acid or a derivative thereof. In particularly preferred embodiments of this type, the intervening one or more atoms are those of lysine, aspartic acid, glutamic acid, ornithine, or 2,4-diaminobutyric acid. Preferred compounds of this type may be represented as

R-X-L-polyamine

wherein R is a straight or branched C10-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy; a C1-8 alicyclic; a single or multiring aryl substituted or unsubstituted aliphatic; an aliphatic-substituted or unsubstituted single or multiring aromatic; a single or multiring heterocyclic; a single or multiring heterocyclic aliphatic; an aryl sulfonyl;

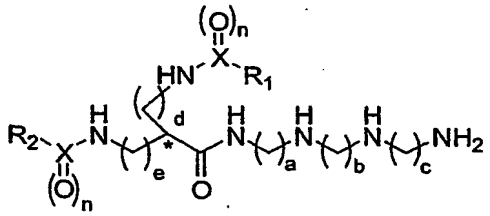
X is -CO-, -SO_2-, or -CH_2-; and

L is a covalent bond or a naturally occurring amino acid, ornithine, 2,4-diaminobutyric acid, or derivatives thereof.

The analogs and derivatives of the invention, may be optionally further substituted at one or more other positions of the polyamine. These include, but are not limited to, internal nitrogen and/or internal carbon atoms. In one aspect of the invention, preferred substituents are structures that increase polyamine transport inhibition, binding affinity or otherwise enhance the irreversibility of binding of the compound to a polyamine binding molecule, such as the polyamine transporter, an enzyme or DNA. Such additional substituents include the aziridine group and various other aliphatic, aromatic, mixed aliphatic-aromatic, or heterocyclic multi-ring structures. Reactive moieties which, like aziridine, bind covalently to a polyamine transporter or another polyamine binding molecule, are also within the scope of this invention. Examples of reactive groups that react with nucleophiles to form covalent bonds include chloro-, bromo- and

iodoacetamides, sulfonylfluorides, esters, nitrogen mustards, *etc*. Such reactive moieties are used for affinity labeling in a diagnostic or research context, and may contribute to pharmacological activity in inhibiting polyamine transport or polyamine synthesis. The reactive group can be a reactive photoaffinity group such as an azido or benzophenone group. Chemical agents for photoaffinity labeling are well-known in the art (Flemming, S.A., *Tetrahedron* **1995**, *51*, 12479-12520).

A preferred aspect of the invention relates to a polyamine analog or derivative that is a highly specific polyamine transport inhibitor with pharmaceutical utility as an anti-cancer chemotherapeutic. One class of a polyamine analog or derivative of the invention that binds to a polyamine-binding site of a molecule and/or inhibits polyamine transport, is described by the following formula II:



wherein a, b, and c independently range from 1 to 10; d and e independently range from 0 to 30; each X is independently either a carbon (C) or sulfur (S) atom, and R_1 and R_2 are as described below, or each of $R_1X\{O\}_n$- and $R_2X\{O\}_n$- are independently replaced by H; and * denotes a chiral carbon position. Where if X is C, then n is 1; if X is S, then n is 2; and if X is C, then the XO group may be CH_2 such that n is 0.

In the above formula, R_1 and R_2 are independently selected from H or from the group of a straight or branched C1-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy; a C1-8 alicyclic; a single or multiring aryl substituted aliphatic; an aliphatic-substituted single or multiring aromatic; a single or multiring aromatic or saturated heterocyclic; a single or multiring heterocyclic aliphatic; a C1-10 alkyl; an aryl sulfonyl; or cyano.

Examples of heterocyclic rings as used herein include, but are not limited to, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, pyrazole, 3-pyrroline, pyrrolidine, pyridine, pyrimidine, purine, quinoline, isoquinoline, and carbazole.

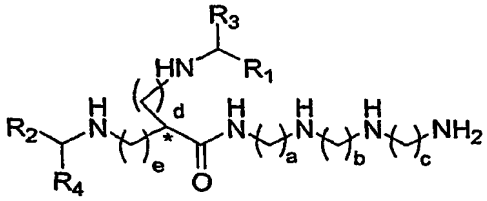
All of the above described aliphatic, carboxyalkyl, carbalkoxyalkyl, alkoxy, alicyclic, aryl, aromatic, and heterocyclic moieties may, of course, also be optionally

substituted with 1-3 substituents independently selected from halo (fluoro, chloro, bromo or iodo), lower alkyl (1-6C) and lower alkoxy (1-6C).

As used herein, carboxyalkyl refers to the substituent -R'-COOH wherein R' is alkylene; and carbalkoxyalkyl refers to -R'-COOR wherein R' and R are alkylene and alkyl respectively. In preferred embodiments, alkyl refers to a saturated straight- or branched-chain hydrocarbyl radical of 1-6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, n-pentyl, 2-methylpentyl, n-hexyl, and so forth. Alkylene is the same as alkyl except that the group is divalent. Aryl or alkyl sulfonyl moieties have the formula -SO_2R, and alkoxy moieties have the formula -O-R, wherein R is alkyl, as defined above, or is aryl wherein aryl is phenyl, optionally substituted with 1-3 substituents independently selected from halo (fluoro, chloro, bromo or iodo), lower alkyl (1-6C) and lower alkoxy (1-6C).

A preferred group of compounds encompassed by the above is where d is 4 and e is 0.

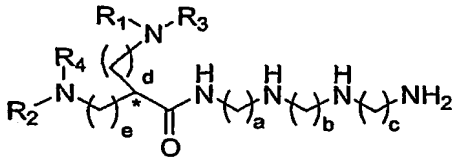
An additional class of a polyamine analog or derivative of the invention that binds to a polyamine-binding site of a molecule and/or inhibits polyamine transport, is described by the following formula III:



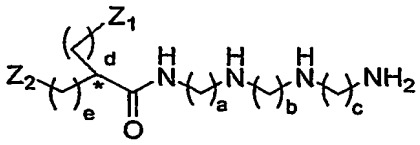
wherein a, b, and c independently range from 1 to 10 and d and e independently range from 0 to 30. R_1 and R_2 are defined as above for formula II and R_3 and R_4 are independently selected from organic substituents including -CH_3 and as defined above for R_1 and R_2 in formula II above. This grouping of analogs is produced by reductive amination of the free amino precursor with a ketone. Some members of this group of analogs are shown in Series V (see Figure 2).

In one preferred embodiment of the invention, R_1 and R_2 are identical and as described for formula II. Positions R_3 and R_4 may also be identical, and all of R_1 through R_4 may also be identical. Additionally, each of positions R_1, R_2, R_3 and R_4 in formula III may also be independently H.

In an additional aspect of the invention the proximal and/or the distal amino group relative to the polyamine (such as spermine) can be di-alkylated to form tertiary amines. These materials can be synthesized by reductive amination with a large excess of the carbonyl component. Additionally, these materials may be produced by a conjugate addition of the amine precursor to an α,β-unsaturated carbonyl or α,β-unsaturated nitrile. Each of R_1, R_2, R_3 and R_4 can be independently varied and are as defined as above for formula III. Each of R_1, R_2, R_3 and R_4 may also be independently H. The values of a, b, c, d and e are as described above for formula III. This aspect of the invention is depicted in the following formula IV:

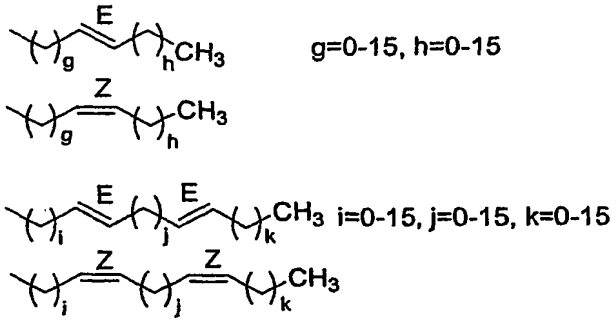


In a further aspect of the invention, compounds which lack the proximal or distal amino group on the acyl portion of the molecule are also provided. These are represented by formula V:



where Z_1 is NR_1R_3 and Z_2 is selected from $-R_1$, $-CHR_1R_2$ or $-CR_1R_2R_3$ (wherein R_1, R_2, and R_3 are as defined above for formula III) or Z_2 is NR_2R_4 and Z_1 is selected from $-R_1$, $-CHR_1R_2$ or $-CR_1R_2R_3$ (wherein R_1, R_2, and R_3 are as defined above for formula III). Values for a, b, and c independently range from 1 to 10; d and e independently range from 0 to 30. Compounds encompassed by formula V may be prepared by first coupling amino acid derivatives (modified to contain the non-amine containing Z group) to a polyamine followed by appropriate derivatization of the amine containing Z group. Chemistries for such reactions are known in the art and disclosed herein.

In preferred embodiments of the invention, positions R_1, R_2, R_3 and R_4 of all the formulas set forth above are independently selected from the following, where each of g, h, i, j, and k are independently selected from 0 to 15:



wherein E refers to "entgegen" and Z refers to "zusammen".

The present invention includes the free base or acid forms, as well as salts thereof, of the polyamine analogs and derivatives described by the above formulas. The invention also includes the optical isomers of the above described analogs and derivatives, especially those resulting from the chiral center indicated above with a *. In a further embodiment of the invention, mixtures of enantiomers and/or diastereoisomers, resulting from a single preparative step, combination, or interconversion are encompassed.

The invention also provides prodrug forms of the above described analogs and derivatives, wherein the prodrug is metabolized *in vivo* to produce an analog or derivative as set forth above. Indeed, some of the above described analogs or derivatives may be a prodrug for another analog or derivative.

In another aspect of the invention, compositions containing the above described analogs and derivatives are provided. Preferably, the compositions are formulated to be suitable for pharmaceutical or agricultural use by the inclusion of appropriate carriers or excipients.

In a further aspect of the invention, methods for the use of the above described analogs and derivatives, as well as compositions, are provided. These methods include uses of the invention's polyamine compounds to inhibit polyamine transport, as well as treat human and agricultural diseases and conditions. Examples of human diseases and conditions include, but are not limited to, cancer, osteoporosis, asthma, autoimmune diseases, rheumatoid arthritis, systemic lupus erythematosus, Type I insulin-dependent diabetes, tissue transplantation, African sleeping sickness, psoriasis, restenosis, inhibition of unwanted hair growth as cosmetic suppression, hyperparathyroidism, inflammation, treatment of peptic ulcer, glaucoma, Alzheimer's disease, suppression of atrial tachycardias,

stimulation or inhibition of intestinal motility, Crohn's disease and other inflammatory bowel diseases, high blood pressure (vasodilation), stroke, epilepsy, anxiety, neurodegenerative diseases, hyperalgesic states, protection against hearing loss (especially cancer chemotherapy induced hearing loss), and pharmacological manipulation of cocaine reinforcement and craving in treating cocaine addiction and overdose and other fungal bacterial, viral, and parasitic diseases. These compounds also find use as agents for use in the trans-cellular delivery of nucleic acids used in anti-sense DNA therapies for numerous disease states. The invention's polyamine compounds may be utilized as, but not limited to being, a soil additive or conditioner in agricultural applications.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows Scheme 1, a pathway for the synthesis of selectively acylated lysine-spermine derivatives. The pathway may be readily adapted for the synthesis of other polyamine derivatives by the use of an analogous protected "NH-X-COO" starting material (wherein X is CH-$(CH_2)_d$-NH-COO-CH_2-Ph, wherein d is as described above and "Ph" is phenyl) and/or the use of any primary polyamine, including spermine.

Figure 2 illustrates exemplary polyamine structures encompassed by the present invention. They have been divided into Series I-VI based upon the character of the chemical moiety attached to a spermine backbone to produce exemplary analogs and derivatives of the invention. Other polyamines may also be used as the backbone. The structures depicted in the first, left-most column of each table represent the specific chemical starting materials utilized in the synthesis of individual polyamine structures. The synthetic steps used result in the end products that are carboxamides from a reaction between an acyl chloride and an amine (series I), sulfonamides from the reaction between a sulfonyl chloride and an amine (series II), carboxamides from the reaction of a DCC, HBTU or PyBOP activated carboxylic acid and an amine (series III), alkylated secondary amines from the reductive amination of the amine with an aldehyde (series IV), alkylated secondary amines with α-alkyl substituents from the reductive amination of the free amino precursor with a ketone (Series V) and di-alkylated tertiary amine products by reductive amination with a large excess of a carbonyl containing (e.g. aldehyde or ketone) component (Series VI). Additionally the Series VI compounds may also be produced by a conjugate

addition of the amine precursor to an α,β-unsaturated carbonyl or α,β-unsaturated nitrile. Columns E and F are directed to doubly derivatized forms of the base chemical structure.

Figure 3 shows representative structures of polyamine analogs relating to the present invention.

Figure 4 shows the relationship between the length of the hydrocarbon substituent at the ε-position of the L-lysine analogs and the resulting activity as polyamine transport inhibitors as defined by EC_{50} (see Example IV).

Figure 5 representatively shows the portion of compounds for calculation of logP values.

Figure 6 presents calculated logP values versus HPLC retention time for dansylated derivatives of compounds shown in Figure 2 (Series I).

Figure 7 presents calculated logP values versus average EC_{50} values obtained for compounds with 4 cell lines (data for Series I compounds in Table 1).

Figure 8 presents HPLC retention time for dansylated derivatives of compounds shown in Table 2 (Series IV and V) versus average EC_{50} values obtained for 4 cell lines (data in Table 1).

Figure 9 shows the relationship between calculated logP values and HPLC retention time for dansylated derivatives of compounds shown in Table 2 (Series IV and V).

Figure 10 presents calculated logP values versus average EC_{50} values obtained for compounds with 4 cell lines (data for Series IV and V compounds in Table 2).

Figure 11 presents HPLC retention time for dansylated derivatives of compounds shown in Table 2 (Series IV and V) versus average EC_{50} values obtained for 4 cell lines using data in Table 1.

Figure 12 shows the structures of exemplary polyamine analogs and derivatives of the present invention.

MODES OF CARRYING OUT THE INVENTION

The present inventors have designed novel polyamine analogs and derivatives for the inhibition of polyamine transport and other uses. These analogs and derivatives are inferred to bind polyamine transporters with high affinity and inhibit polyamine transport,

either competitively or non-competitively. Thus these compounds can alter polyamine metabolism in cells by reducing or preventing polyamine uptake.

In particularly preferred embodiments of the invention, one or more polyamine analogs and derivatives are used in combination with polyamine synthesis inhibitors to inhibit cell growth and proliferation. As such, they are useful as drugs in a number of diseases, particularly cancer and other conditions involving cellular proliferation, including, but not limited to, inflammatory diseases or conditions where components of the immune system undergo undesired proliferation. Non-limiting examples include asthma, autoimmune diseases, rheumatoid arthritis, systemic lupus erythematosus, Type I insulin dependent diabetes, psoriasis, restenosis, inhibition of unwanted proliferation of hair on skin, tissue transplantation, African sleeping sickness, osteoporosis, hyperparathyroidism, treatment of peptic ulcer, glaucoma, Alzheimer's disease, suppression of atrial tachycardias, stimulation or inhibition of intestinal motility, Crohn's disease and other inflammatory bowel diseases, high blood pressure (vasodilation), stroke, epilepsy, anxiety, neurodegenerative diseases, hyperalgesic states, the protection of hair cells from chemotherapy induced loss of hearing, and pharmacological manipulation of cocaine reinforcement and craving in treating cocaine addiction and overdose, and other fungal, bacterial, viral, and parasitic diseases.

As used herein, the term "polyamine" includes putrescine, spermine or spermidine, as well as longer linear polyamines, branched polyamines, and the like, which may have between 2 and about 10 nitrogens. Also included in this definition are polyamine derivatives or analogs comprising a basic polyamine chain with any of a number of functional groups bound to a C atom or a terminal or internal N atom. For modification at a primary amino group, a polyamine must, of course, contain such a group.

Polyamine "analogs" and/or "derivatives" generally refer to any modified polyamine molecule disclosed or described herein. These molecules are generally modifications of existing polyamines, whether naturally occurring or synthetically produced, and may also be referred to as "polyamine agents", "PA" or "agents" of the invention. Preferred PAs bind and/or inhibit cellular polyamine transport, and as such may also be referred to as "transport binding molecules" or "polyamine transport inhibitors". The scope of this definition includes any modification to produce a PA from an existing polyamine or the isolation of a structurally identical PA from a naturally occurring source.

Preferably, the modification is the addition of one or more chemical moieties to the polyamine.

A PA that is an "inhibitor" polyamine analog or derivative (a) binds to polyamine transporters better than a native polyamine and/or (b) by some means blocks the uptake of a polyamine into a cell or a subcellular polyamine transporter preparation. The invention includes PAs that efficiently inhibit polyamine transporters in different eukaryotic cell types as well as inhibit cellular growth and proliferation when used in combination with a polyamine synthesis inhibitor.

The PAs of the invention generally have an acylated primary amine functionality and are expected to bind to a cell's polyamine transporter apparatus with a very high affinity. Measurements of K_i were determined by using an assay that shows the inhibition of polyamine uptake, such as uptake of ^{3}H-spermidine.

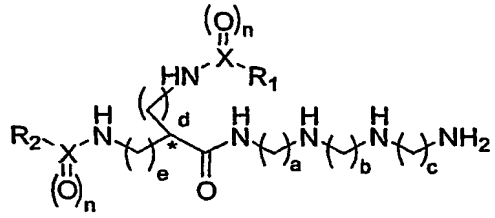
The PAs were also analyzed with a secondary assay to show inhibition of cellular polyamine uptake based on a measurement of cellular growth inhibition in combination with a potent inhibitor of polyamine biosynthesis. This assay was conducted in the presence of polyamines, such as spermidine, to determine a PA's ability to prevent the uptake of polyamines thereby overcoming the polyamine biosynthesis inhibition with DFMO (difluoromethylornithine). Due to the trend that polyamine mono-amides give high potency in both of these assays, it has been inferred, without limiting the invention thereto, that there is a site on the transporter protein for tight binding of the inhibitor's amide functionality.

Preferred embodiments of these PAs are the result of acylation at a polyamine molecule with two or more primary amine groups. The linkage between the acyl group and the primary amine group is preferably an amide linkage (indicated below as the bond between "CO" and "NH") and results in a molecule with the following general formula.

rest of acyl group-CO-NH-rest of polyamine

As noted above, other linkages, whether direct or indirect, may also be used. The "polyamine" in the above formula may be any polyamine with at least one primary amine group, but more preferably with two or more primary groups, for linkage to the acyl group.

One preferred class of acyl groups for inclusion in the above formula contains two primary amines for further acylation. The resultant class of PAs may be described by the following formula (formula II).



as defined above. Non-limiting examples of alkyl moieties as present in these compounds include straight or branched chains of at least about 8 carbon atoms for increased hydrophobicity (or lipophilicity), such as at least about 10, at least about 12, at least about 14, at least about 16, at least about 18, at least about 20, at least about 22, at least about 24, at least about 26, at least about 28, and at least about 30. In yet another set of preferred embodiments, the chain is of at least about 19, 21, 23, 25, or 27 carbon atoms, with at least about 20 to at least about 24 or 26 as even more preferred.

A particularly preferred group of PAs encompassed by the above formula is where d is 4 and e is 0, although generally excluded from this group are PAs where $R_2X\{O\}_n$- is an H and $R_1X\{O\}_n$- is R_1SO_2- wherein R_1 is a thiophene moiety linked to the S atom via the 2 position, and substituted at the 5 position, of the thiophene. Preferably excluded are such PAs wherein the substitution at the 5 position includes an amide linkage. Also preferably excluded are such PAs wherein the amide linkage is attached to a chlorinated aromatic group, such as the compound identified as ORI 1340 in U.S. Patent Application Serial No. 09/396,523, filed September 15, 1999.

Other classes of PAs as encompassed by the invention are set forth as formulas I, III, IV, and V as described above. In all of the formulas of the invention, the term "single or multiring alicyclic" includes adamantyl type structures. Moreover, the term "substituted" used in conjunction with the description of any chemical moiety for a formula of the invention includes the attachment of the moiety to the rest of the formula by way of the "substitution". The term also indicates that "unsubstituted" forms of the described chemical moiety is also within the scope of the invention.

By analyzing the relationship between a polyamine analog's structure and its ability to act as a polyamine transport inhibitor, it was discovered that increases in the lipophilic

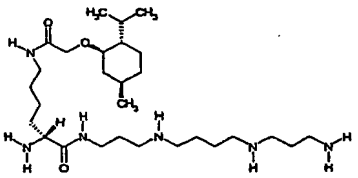
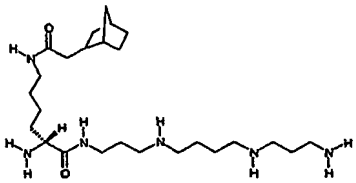
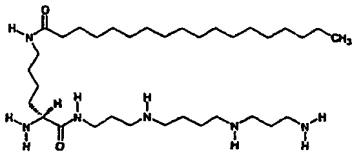
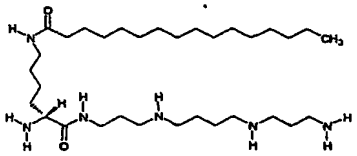
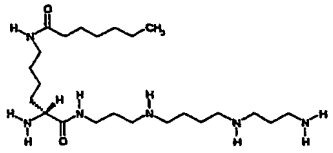
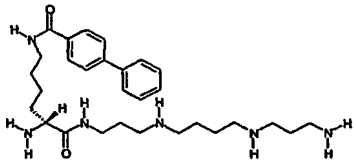
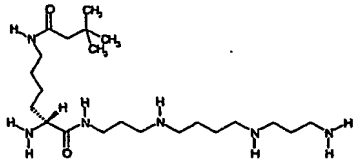
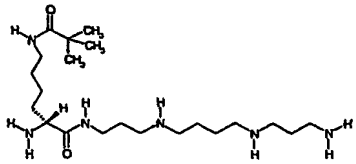
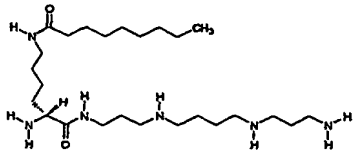
character of the hydrophobic substituent on the polyamine may increase transport inhibition. While the nature of the interaction between a lipophilic polyamine analog and the polyamine transport apparatus remains unclear at this time, the invention includes, but is not limited to, situations where the hydrophobic (lipophilic) moiety may serve as an anchor to some hydrophobic pocket on the transporter or in a region nearby. This may result in the interaction of the polyamine portion of the analog with the polyamine transporter.

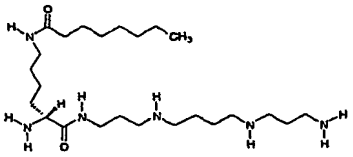
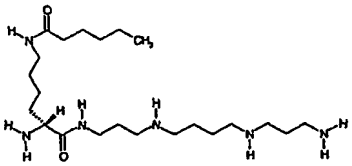
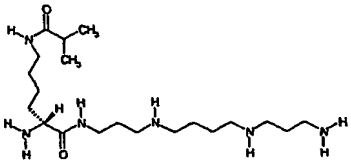
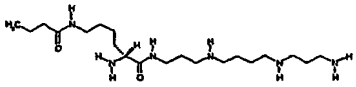
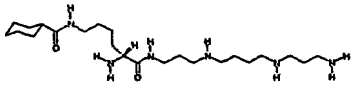
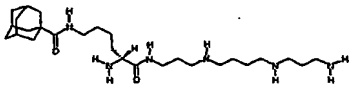
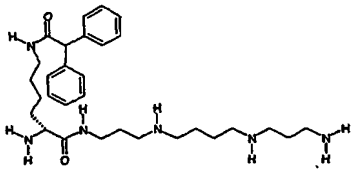
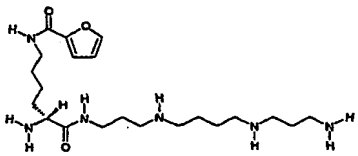
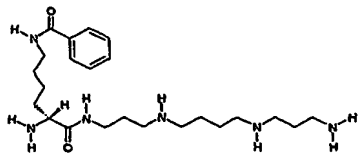
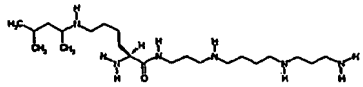
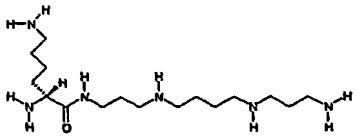
There are a number of ways one might analyze the hydrophobic character of compounds described in the present invention. The following two scales describe ways to measure relative degrees of lipophilicity.

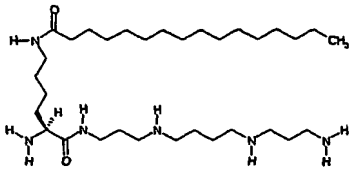
The logP coefficient is the logarithm of the ratio of distribution of a compound in a mixture of 1-octanol and H_2O. Compounds with logP values greater than 1 are considered lipophilic (greater solubility in 1-octanol versus H_2O). The presence of ionizable groups in the compound has a dramatic effect on this parameter. Ionization will greatly increase a compound's H_2O solubility. For this reason, a compound's ionization potential must be taken into consideration when correlating lipophilicity with activity. One can use a variety of computerized protocols to perform calculated estimates of the logP value. One such computer program is ChemDraw Pro Version 5.0 from CambridgeSoftCorporation. One of the several methods that this program uses to calculate the logP coefficient is through Crippen's fragmentation method (Crippen et. al., *J. Chem. Inf. Comput. Sci.* **1987**, *27*, 21). The present invention used this method to calculate logP values for fragments of the described molecules. These fragments were generated in the fashion depicted in Figure 5. The results of these calculations are provided in Table 1 for the D-stereoisomers of the ε-acyl substituted Lys-spm conjugates (Figure 2, Series I) and in Table 2 for the D-stereoisomers of the ε-alkyl substituted Lys-spm conjugates (Figure 2, Series IV and V).

Table 1: Chemical structure (with ID relative to Figure 2), logP Calculations, HPLC data and average EC_{50} values for D-stereoisomers of ε-acyl-substituted spermine based analogs (Figure 2, Series I). Compound 1426 and one Series V compound are included for comparison.

ID	Structure	LogP	Ret Time - Std	Ave EC_{50} value

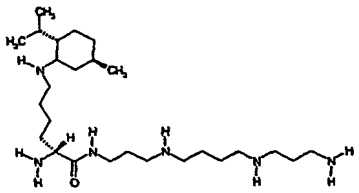
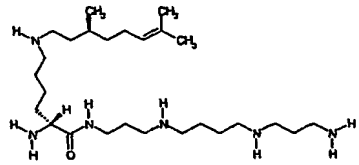
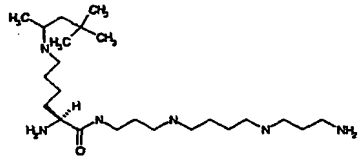
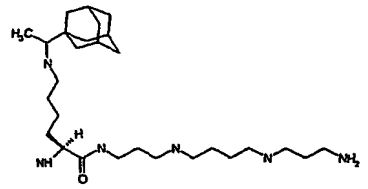
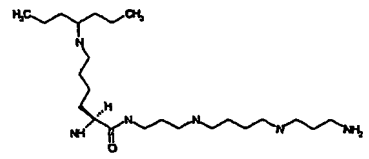
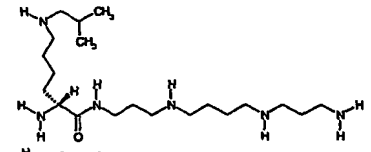
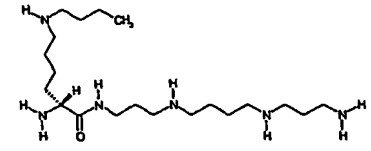
IB38		1.73	9.63	13
IB37		1.03	6.33	41
IB2		6.59	21.1	0.083
IB4		5.68	15.82	0.084
IB8		1.57	6.07	3.5
IB26		2.01	6.34	1.1
IB36		1.21	4.91	27
IB34		0.75	4.6	8.5
IB6		2.48	10.48	2.2

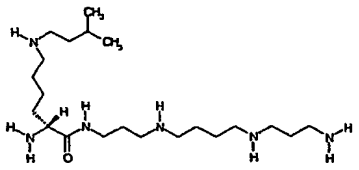
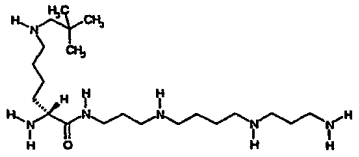
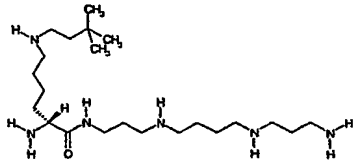
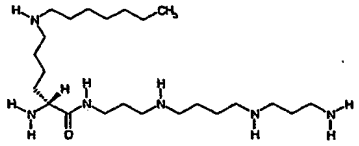
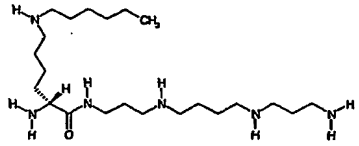
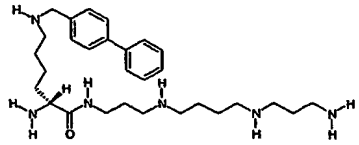
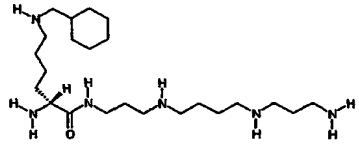
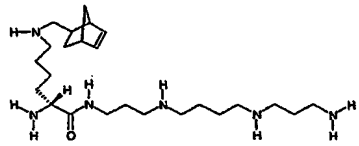
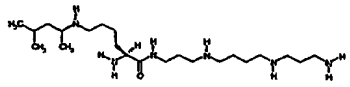
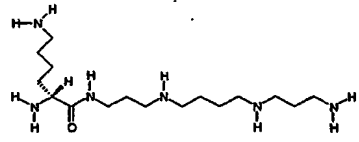
IB7		2.03	6.83	13
IB9		1.12	5.16	12
IB33		-0.05	3.56	8.4
IB10		0.2	3.46	12
IB32		0.97	5.29	3.6
IB30		1.68	7.4	2
IB29		1.99	6.08	2.1
IB25		-0.44	No Data	10
IB24		0.58	4.23	30
VA21		1.04	10.11	0.65
1426		Not calc'd	6.68	3.7

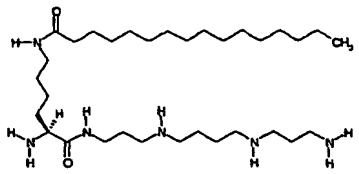
IA4		5.68	15.79	0.13

Preferred PAs of the invention with respect to Series I type compounds are those with low EC_{50} values, such as those with below about 5, about 6, about 7, about 8, about 9, about 10, about 15, about 20 or about 25 minute HPLC retention times.

Table 2: Chemical structure (with ID relative to Figure 2), calculated logP value, HPLC retention time, and average EC_{50} value for ε-alkylated spermine based analogs (Figure 2, Series IV and V). Compound 1426 and one Series I compound are included for comparison.

ID	Structure	LogP	Ret Time-Std	Ave EC_{50} Value
VB28		2.01	13.89	1.45
IVB28		2.21	9.4	12.8
VA22		1.84	10	2.42
VA27		2.31	12.71	26.8
VA26		1.74	10.84	4.14
IVB23		0.66	9.05	1.79
IVB3		0.91	9.16	2.19

IVB21		1.12	9.62	1.32
IVB24		1.46	9.35	1.32
IVB22		1.92	9.85	0.68
IVB6		2.28	10.87	0.89
IVB5		1.83	10.27	0.71
IVB33		2.45	10.01	1.38
IVB27		1.68	10.31	0.61
IVB25		0.57	9.89	0.89
VA21		1.04	10.11	0.65
1426		Not calc'd	6.68	3.68

IA4		5.68	15.79	0.13

Preferred PAs of the invention with respect to Series IV and V type compounds are those with low EC_{50} values, such as those with below about 5, about 6, about 7, about 8, about 9, about 10, about 12, about 14, about 16, about 18, or about 20 minute HPLC retention times.

Another way to measure relative hydrophobicity would be chromatographic techniques such as comparison of HPLC retention times on C18 reverse phase columns, longer retention times would represent greater relative hydrophobicity. The present invention utilized a dansylation protocol to form dansyl derivatives of the described analogs and analyzing these derivatives by fluorescence detection on C18 reverse phase HPLC. The difference between the elution of the peak due to the analog and the peak due to an internal standard (1,7-diaminoheptane) is shown for several representative analogs in Tables 1 and 2 above.

The relationship between calculated logP values and the HPLC retention time of the dansylated derivatives are plotted in Figures 6 and 9 for Series I and IV type compounds, respectively. The relationship between calculated logP and average EC_{50} values are plotted in Figures 7 and 10 for Series I and IV type compounds, respectively. The relationship between HPLC retention times and average EC_{50} values are plotted in Figures 8 and 11 for Series I and IV type compounds, respectively.

An additional compound hydrophobicity scale, specific for amino acids, was devised and measured by R. Wolfenden (Wolfenden, R.; Andersson, L.; Cullis, P.M.; Southgate, C.C.B. Affinities of amino acid side chains for solvent water. *Biochemistry*, **1981**, *20*, 849-855.). They measured the equilibria of distribution of amino acid side chains between their dilute aqueous solutions and the vapor phase. They describe a scale of "hydration potentials" whereby buffered H_2O-vapor phase distribution measurements were made on the side-chain portions of the amino acids (e.g. methane for alanine, methanol for serine, n-butylamine for lysine or n-propylguanidine for arginine). If a side-chain had the potential for ionization a correction was made such that only the un-ionized fraction was considered. This was based on calculation of the un-ionized fraction using literature pKa

values. The side chains for the twenty naturally occurring amino acids span a range of free energy values for the transfer from the vapor phase to H_2O from 2.39 kcal/mol for hydrogen (glycine) or 1.94 kcal/mol for methane (alanine) to –7.00 kcal/mol for n-butylamine (lysine) or –14.6 kcal/mol for n-propylguanidine (arginine).

These values form a "hydration potential" scale, which is correlated with the potential that a given amino acid would be present on the outside, or hydrophilic portion of a protein versus the more hydrophobic interior of a protein. The authors state "that the energetic cost of removing hydrophilic side chains from water is much greater than the cost of pulling hydrophobic side chains into water, and, indeed, it has been observed that hydrophobic residues occur rather often at the surfaces of proteins." The present invention could use this scale to describe the lipophilicity of the substituent attached to the polyamine. The polyamine portion is removed before this analysis. As an example, it is also required that the α-amino and α-carboxylate groups of any analogs containing an α-amino acid be removed before analysis. By using this scale, any substituent with a free energy of transfer from the vapor phase to H_2O less than that determined for n-butylamine (and thus correlated to lysine) of –7.00 kcal/mol would be expected to be a preferred polyamine transport inhibitor in comparison to the lysine-spermine conjugate (ORI 1202). This means any substituent that gives a hydration potential greater (more positive) than -7.00 kcal/mol, as defined in this scale, results in polyamine transport inhibitors with significant activity (values of free energy of transfer which are more negative mean a given compound would have a greater solubility in H_2O than the vapor phase).

The preferred group of PAs wherein d is 4 and e is 0 includes both the L and D-stereoisomers due to the chiral carbon indicated by * in the above formula. Exemplary PAs such as ORI 1202 (L-Lys-spm), 1426 (D-Lys-spm), and those containing IA4 (Figure 2) demonstrated potency in both the transporter inhibition and cell growth inhibition assays described below. PA ORI 1202 also displayed effectiveness in several anti-cancer mouse xenograft models. See Weeks, R.S., Vanderwerf, S.M., Carlson, C.L., Burns, M.R., O'Day, C.L., Cai, C.F., Devens, B.H., and Webb, H.K. *Exp. Cell Res.* **2000**, *261*, 293-302. and Devens, B.H., Weeks, R.S., Burns, M.R., Carlson, C.L., and Brawer, M.K. Prostate Cancer and Prostatic Diseases **2000**, *3*, 275-279.

Additional modification of the two primary amine groups in the acyl group in the above formula is readily accomplished by the availability of the primary amine groups for selective functionalization together with the commercial availability of orthogonally di-

protected versions of $H_2N(CH_2)_nCH(NH_2)COOH$ type molecules (where n ranges from 1 to 50 for example), such as lysine and ornithine.

Without being bound by theory, increases in the lipophilicity of the substituent at the above R_1 and R_2 positions may dramatically increase the affinity for the polyamine transporter. Increases in lipophilicity in the PAs of the invention may improve the inhibition of polyamine transport due to the presence of both hydrophilic and hydrophobic domains. Biological systems have a significant chemical problem when they attempt to move a very hydrophilic substance, such as polycationic polyamines, across their very hydrophobic outer membrane barriers. If the transporter moves the polyamines in their polycationic forms across this barrier, the transporter may do so via some mechanism for masking or minimizing their hydrophilicity. Mechanisms for this may include the formation of specific salt bridges between the polyamine and negatively charged residues on the protein or formation of a charged interior in the intermembrane pore. Because polyamine transport is known to be an energy dependant process, the transporter may have the task of providing a very specific polyamine shaped hydrophilic pore in the presence of the very hydrophobic environment of the membrane. For these reasons the transporter likely has hydrophobic residues for interactions with the membrane in close proximity to hydrophilic residues specific for interactions with the polyamine.

By designing PAs that contain both hydrophobic and hydrophilic domains, the present invention exploits the likely characteristics of a polyamine transporter to improve transport inhibition. Thus the present invention provides several series of PAs that contain both a polyamine-mimicking portion and a hydrophobic membrane-mimicking portion. These PAs have been inferred to have great affinity for the transporter, and they show substantially increased growth inhibition (in combination with a polyamine synthesis inhibitor) in comparison to PAs lacking a significantly hydrophobic domain. Probably for very similar reasons, the present PAs are also expected to show improved bioavailability through oral administration. Increases in lipophilicity are expected to enhance absorption after oral uptake.

It is also expected that the introduction of both hydrophilic and hydrophobic domains in the same molecule, as shown by those in the present invention, will also enable them to facilitate the transfer of nucleic acids through biological membranes. This property gives the analogs usefulness as transfer agents for anti-sense DNA for a number of scientific, analytical, diagnostic and therapeutic applications.

The above is supported by analysis of the results of extending a straight-chain aliphatic saturated hydrocarbon at position R (see Figure 2, Series I) results in increases in cell growth inhibition in the presence of a polyamine synthesis inhibitor. The clear trend that longer hydrocarbon chains on this amide position increase potency is indicated by a comparison of spermine based compounds IA4, IA8, and IA11 as well as IB4, IB7, and IB8 (see Table 3). Figure 4 shows the relationship between the length of the hydrocarbon substituent at the R position and the resulting EC_{50} value in the presence of a polyamine synthesis inhibitor.

Table 3 shows the results from analysis of various exemplary PAs for their ability to inhibit cellular growth in combination with DFMO relative to control cells left untreated. EC_{50} refers to the concentration of PA resulting in 50% of maximum cell growth inhibition in the presence of both DFMO and the PA. K_i refers to the inhibition constant for polyamine transport based on double reciprocal Lineweaver-Burke plot analyses of four radioactive substrate concentrations (0.3-3 μM) and five inhibitor concentrations (0.01-1.0 μM) and a control. Compounds ORI 1202 and 1426 are included for comparison. See the Examples below.

Table 3: EC_{50} values (μM) of representative polyamine analogs (see Figure 2) determined in the presence of DFMO (1-5 mM). Also shown are the IC_{50} results from analyses of various exemplary PAs. IC_{50} refers to the concentration of PA that results in 50% of maximum cell growth inhibition in the presence of PA alone.

Analog	Cell Line EC_{50} (μM)				AVG. EC_{50}	Cell Line IC_{50} (μM)				K_i
	A375	MDA-MB-231	PC-3	SK-OV-3	(μM)	A375	MDA-MB-231	PC-3	SK-OV-3	(μM)
IA40		29.8	7.87				>300	>300		0.039
		41.3	8.51				>300	>300		
IC41		36.9	16.9				>300	430		0.191
1202	1.49	4.75	5.3	0.5	4.542		>300	560		0.031
		2.5	1.7	0.51						
		2.5	1.24							
		13.5	1.24							
		6.9	10.3							
		8.7	0.822							
		8.4	7.78							
		4.35	4.1							
			6.2							
			2.6							
IVE30		4.2	1.7							
IIA21		1.4	0.46							
IB41		31.9	6.73							
1426	1.91	4.5	5	0.51	2.254	1620	1840	1840	2530	0.034
	1.29	1.5	8.02	0.93		>100	>100	>100	>100	
	2.2	1.27	0.55	6.09		>300	>300	>300	>300	
	1.75	4.25	2.12	1.36		>100	>300	>300	>300	
	0.829	2.02	0.704	1.41		>100	>100	>100	>300	
	2.7	1.27	0.52	0.53		>100	>100	>100	>100	
		2.1	0.26	2.7			>100	>100	>100	
		3.99	0.89	>100				>100	>100	

		3.1	2.98 4	0.68 2.7					>100	
IIA20	0.405	1.61	0.463	2.65	1.282	>30	>30	>30	>30	
IA4	0.049 0.049 0.008 0.005 0.004 <0.1	0.194 0.057 0.017 0.005 0.009 <0.1	0.129 0.028 <0.001 0.001 <0.1	0.273 0.069 0.252 0.049 <0.1 0.182	0.077	>30 61.5 >3 >3 >3 58.3	>30 62.4 >3 >3 >3 62	>30 >3 >3 >3 18.1	>30 >3 >3 >3 18.6 >3	0.0015
IA28	1.66	>30	0.982	>30		>30	>30	>30	>30	
IA19	0.214	>30	>30	>30		>30	>30	>30	>30	
IA11	>30	>30	2.3	>30		>30	>30	>30	>30	
IB4	0.071 <0.01 0.026 0.015 <0.1 0.011	0.168 <1 0.031 0.072 0.051 <0.1 0.06	0.197 0.044 0.177 0.09 <0.1 0.116	0.297 0.121 0.175 0.121 <0.1 0.157	0.105	>30 23.1 >3 >3 55.4 >3	>30 58.9 >30 >3 >3 56 >3	>30 26.1 >3 >3 15.8 >3	>30 27.7 >3 >3 12.6 >3	0.017
IIA17	0.629	<1	0.18	2.59		>30	605	>30	>30	
IIA2	>30		>30	>30		>30		>30	>30	
IA7	2.3 1.75	1.12	1.35 0.853	>30 30	6.229	>30 >30	>30	>30 >30	>30 >30	
IA24	1.56		>30			>30		>30		
IB24	>30	>30	>30	>30	>30	>30	>30	>30	>30	
IB7	2.61 4.87	>30 19	1.27	>30 28.3	11.210	>30 >30	>30 >30	>30	>30 >30	
IIB2	7.25		3.64	>30		>30		>30	>30	
ID24	5.98	4.75	3.3	>30		>30	>30	>30	>30	
ID7	5.29	8.25	7.42	17.2	9.540	>30	>30	>30	>30	
IID17	5.87	5.1	4.09	23.9	9.740	>30	>30	>30	>30	
IID2	>30	>30	>30	>30	>30	>30	>30	>30	>30	
ID25	8.78	8.76	5.27	>30		>30	>30	>30	>30	

ID4	0.44	0.636	1.33	2.64	1.262	17.9	18.6	18.7	18.1	
IB25	4.27		3	22.9		>30		>30	>30	
IIB10	0.026	0.169	0.099	0.134	0.110	18	>30	22	18.1	0.002
	0.044		0.074	0.224		17.7		19.9	23.1	
IB6	1.85		1.93	2.84		>30		>30	>30	0.075
IIB17	1.52		0.919	26.2		>30		>30	>30	
IIA10	0.016	0.364	0.024	0.098	0.072	18.3	>30	19	26.7	0.004
	0.01	0.052	0.039	0.083		18.4	>30	17.1	24.1	
	0.009	0.022	0.071	0.08		>3	>3	>3	>3	
IIIA1	0.076	0.197	0.386	0.398	0.264	>30	>30	>30	>30	
IIIB1	0.17	0.491	0.099	1.57	0.583	>30	>30	>30	>30	0.054
IVA18	0.05	0.107	0.075	0.14	0.079	>30	>30	>3	>3	
	0.061	0.038				>3	>3			
IA1	0.01	0.016	0.014	0.083	0.017	18.5	15.3	>3	>3	0.015
	0.004	0.012	0.005	0.02		>3	>3	>3	>3	
	0.002	0.003				>3	>3			
IIIA5	0.084	0.207				>30	>30			
IA3	<0.01	0.032	0.022	0.097	0.053	23	>30	18.3	>30	
	0.01	0.018	0.022	0.167		>3	>3	>3	>3	
IIIA4	0.014	0.039	0.056	0.134	0.061	17.3	>30	23.1	>30	
IA2	<0.01	0.019	0.016	0.027	0.014	13	27.3	13.3	16.8	0.0014
	0.002	0.006	0.007	0.021		>3	>3	>3	>3	
IA5	0.025	0.208	0.189	4.6	1.256	>30	>30	9.87	21.5	
IIA16	1.21	2.57	0.72	>30		>30	>30	>30	>30	
IIIA3	0.017	0.03	0.029	0.082	0.040	>30	>30	>30	>30	
IIIA6	0.018	0.047	0.06	0.095	0.055	22.3	>30	25.8	>30	
IIIA2	0.01	0.029	0.022	0.076	0.034	>30	>30	>30	>30	
IVA11	0.01	0.019	0.046	0.081	0.039	>3	>3	>3	>3	
IIE10		0.392	0.152	0.272			24.5	14.3	20.1	
IE4		0.267	0.2	0.132			17.9	21.5	7.25	
IB2	0.016	0.028	0.091	0.198	0.083	>3	>3	>3	>3	
IIIA7	0.087	0.215	0.255	2.94	0.874	>3	>3	>3	>3	

VA21	0.167	0.392	0.83	1.86	1.296	>300	>300	>100	>300	
	0.141	0.85	0.654	2.3		>300	>100	>100	>100	
	0.63	1.377	0.6	3.669		>100	>100	>100	>100	
	0.498	1.3	2.5	2.3		>100	>100	>100	>100	
	0.48	1.6		3.1		>100	>100		>100	
	0.67					>100	>100			
IVB25	0.32	0.59	0.33	1.75	0.939	>300	>100	>100	19	
	0.4	0.93	0.59	2.6		61	61	>100	>100	
IVB27	0.14	0.39	0.58	0.87	0.414	>300	>100	>100	33.9	
	0.17	0.14	0.12	0.9		>100	>100	>100	>100	
IVB33		1.46	0.77	1.91			>100	>100	72.9	
IB29		3.38	0.56	2.41			>100	>100	>70	
IVB5	0.53	0.224	0.295	1.65	0.868	>100	>100	>100	>100	
		0.9	0.58	1.9			>100	>100	>100	
IVB6	0.17	0.193	<0.1	0.478	0.365	>100	>100	>100	>100	
		0.34	0.18	0.83			>100	>100	>100	
IVB22	1.2	0.194	0.25	1.553	1.335	>100	>100	>100	>100	
	1.95	0.56	1.2	2.6		>100	>100	>100	>100	
		2.08	0.57	2.53			>100	>100	>100	
IB30	0.35	2.4	0.58	4.7	1.244	>100	>100	>100	>100	
	0.21	0.55	0.7	0.46		7.4	84.4	18.8	17.8	
IB32	0.67	4.4		5.6		>100	>100	>100	>100	
XXX*	2.76	6.761	6.218	24.1	9.960	>100	>100	>100	>100	
IB10	3.633	5.962	8	29.091	11.672	>100	>100	>100	>100	
IVB24	0.625	0.961	0.975	2.732	3.138	>100	>100	>100	>100	
	0.51	1.4	15.6	2.3		84.4	>100	18.8	>100	
IVB21	0.526	0.653	1.454	2.7	1.522	>100	>100	>100	>100	
	0.5	0.87	0.87	4.6		71	>100	>100	>100	
IVB3	0.753	1.615	1.657	4.791	2.204	>100	>100	>100	>100	
IVB23	0.636	1.636	1.139	3.788	1.787	>100	>100	>100	>100	
	0.7	1.8	2	2.6			>100	>100	>100	
IB33	2.649	4.726	6.408	20.526	8.577	>100	>100	>100	>100	
IB9	4.4	14.1	3.92	23.5	11.480	>100	>100	>100	>100	

IB34	6.25	11.4	1.93	13.6	8.295	>100	>100	>100	>100	
IB36	6.69	25	2.24	73.7	26.908	>100	>100	>100	>100	
IB26	0.51	0.93	0.46	2.32	0.955	>100	>100	>100	>100	
	0.22	0.6	0.8	1.8		24.6	>100	>100	>100	
IB8	2.6	1.25	2.16	8.18	3.548	>100	>100	>100	>100	
IB35	>100	>100	>100	>100	>100	>100	>100	>100	>100	
VA26	1.44	4.5	1.9	7.8	3.910	>100	>100	>100	>100	
VA27	3.7	12	1.6	8.5	6.450	>100	>100	>100	>100	
VA22	0.79	1.3	0.67	4.7	6.983	>100	>100	>100	>100	
	0.9	2.4	2	43.1		83.5	>100	>100	>100	
IVB28	4.9	6.4	13.5	18.1	10.725	5.6	18.9	17.8	19.8	
IB37	18.3	17.8	39.3	65	35.100	19.8	>100	>100	18.3	
IB38	1.08	17.3	2.4	32.7	13.370	21.3	63.9	28	60.1	
VB28	0.45	0.41	0.75	2.4	0.905	>100	>100	>100	>100	
	0.3	0.43	0.8	1.7		64.5	>100	>100	>100	
IA25										
VIA21		0.68	0.19	>100			>100	>100	>100	
VIB22		0.38	5.49	>100			30	>100	>100	
IB39		52.5	>100	>100			4.26	>100	>100	
IVA6										
IVB26	2.4	1.99	0.91	7.56	3.410	>100	>100	>100	>100	
			1.53	6.07				>100	>100	
VIB26	4.43	8.04	1.58	17.32	7.843	>100	>100	>100	>100	
IVF27	2.18	2.34	0.5	2.16	1.795	>100	>100	>100	>100	
IVF6	0.94	8.03	1.88	9.5	5.088	67.89	>100	>100	67.69	
IVA25	1.04	3.55	0.71	2.3	1.900	>100	>100	>100	>100	
IVA27	0.94	1.32	0.62	0.71	4.691	>100	>100	>100	>100	
	5.06	8	1.88	19		>100	>100	>100	>100	
IVA6	0.54	0.51	0.29	0.24	0.395	>100	>100	>100	>100	
IVA22	0.739	1.66	0.711	0.937	1.012	>100	>100	>100	>100	

*shown in Figure 12.

A set of PAs wherein positions R_1 and R_2 of formula I are substituted by an aliphatic chain with varying degrees of unsaturation in the hydrocarbon chain are represented in Figure 2, Series III. These compounds include those with internal geometrically cis (zusammen or Z-form) and trans (entgegen or E-form) isomers are also presented in this series.

In addition to lipophilicity effects, the invention incorporates considerations based on the charge character of the PA. As obvious from the above general formula II for PAs of the invention, the introduction of the $R_1X\{O\}_n$- and $R_2X\{O\}_n$- moieties reduces the number of positive charges in the analog or derivative by one. At physiological pH of 7.2 the vast majority of amine groups will be in their positively charged ammonium state. The importance of positive charges for inhibiting polyamine transport is suggested by the observation that a PA with acetamide (IA11) showed a higher EC_{50} in comparison to analogous PAs wherein both $R_1X\{O\}_n$- and $R_2X\{O\}_n$- are replaced by hydrogen atoms (see IA11 versus ORI 1202 and ORI 1426 in Table 3).

Series IV (see Figure 2) incorporates the above considerations for both lipophilicity and positive charges by incorporating both a long hydrocarbon chain and retaining the positively charged ammonium function. The reductive amination used to produce these structures results in alkylated (instead of acylated) amines. These compounds are inferred to have great affinity for the polyamine transporter. PAs with a dimerized spermine structure, represented by structures such as IA19, showed no improvement over the original lysine-spermine conjugate.

An alternative group of PAs, based on the long-chain hydrocarbon containing carboamides (Figure 2, Series I), may be prepared by incorporating the lipophilic and biologically stable sulfonamide group. These PAs are shown in Figure 2, Series II. Without being bound by theory, it may be that the addition of an additional carbonyl-like oxygen atom in the sulfonamide series increases the interactions at an amide-binding domain of polyamine transporters. An additional factor which may be playing a role is the increased lipophilicity in sulfonamides versus carboxamides. Additionally sulfonamides are known to be more biologically stable in comparison to carboxamides.

The present invention also provides additional ways to increase the lipophilicity of the substituents on the PA molecule. Alternatives with additional alkyl groups on the acyl portion of the molecule will increase the lipophilicity of this group and thus give an analog with higher activity. One additional method to increase this lipophilicity is through

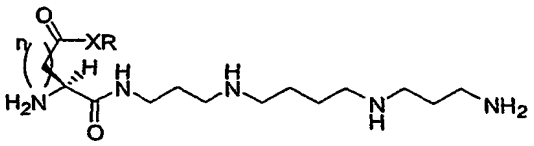
attachment of an additional alkyl chain alpha to the amino group (substituent which is attached to the carbon atom attached to the nitrogen). These analogs are produced by reductive amination of the free amino precursor with one of the ketone reagents shown in Series V. An additional advantage provided by inclusion of a methyl, or other substituent, at the alpha position of the amine group is decreased rate of biological metabolism.

An additional method to increase the lipophilicity of the analogs is through the production of a tertiary amine at the proximal or distal, or both, nitrogen atoms of the molecule. These molecules, which are shown in Series VI, are produced via the reductive amination reaction using a free mono- or di-amine precursor and an excess of the carbonyl containing reagent shown in Series VI. An alternative method to produce these di-substituted tertiary amine containing molecules is the conjugate addition of the selectively protected amine precursor to an α,β-unsaturated carbonyl compound or an α,β-unsaturated nitrile compound.

The present invention further provides methods for the synthesis of the disclosed PAs. In general, an orthogonally protected diamine containing compound, such as, but not limited to, certain amino acids, is coupled to a primary amine group of a polyamine followed by deprotection of one or both of the protected amine groups followed optionally by further derivatization of the amine. Without limiting the scope of the invention, an exemplary scheme for the production of spermine based PAs according to the above formula wherein d is 4, e is 0, X is C, and either $R_1X\{O\}_n$- or $R_2X\{O\}_n$- is H is shown in Figure 1, where the 4-nitrophenyl activated ester Boc-*L*-Lys-(Cbz)-ONP is used in combination with spermine. This scheme is for illustrative purposes only, and any other diamino containing amino acid including, but not limited to, *D*-lysine, *L*-ornithine, *D*-ornithine, *L*-2,4-diaminobutyric acid, *D*-2,4-diaminobutyric acid, *L*-2,3-diaminopropionic acid and *D*-2,3-diaminopropionic acid can be likewise orthogonally di-protected and coupled to spermine. Any appropriate protecting group(s) may be used in the practice of the invention, and the indication of Boc- (butoxycarbonyl-) and Cbz- (carbobenzoxy-) protecting groups are for illustrative purposes only. Other protective group strategies are known in the art (see, for example, "Protective Groups in Organic Synthesis – Third Ed. 1999, eds. T.W. Greene and P.G.M. Wuts. John Wiley and Sons, Inc. New York).

In another aspect of the invention, polyamine analogs may be prepared via the coupling of distal carboxylic acid containing amino acids with suitable protecting groups on this distal carboxylic acid (e.g. methyl or benzyl ester) such as *N*-^{t}Boc-Asp(OCH_3)-OH

or *N*-^{t}Boc-Glu(OCH_3)-OH with a primary amine group of a polyamine (such as, but not limited to, spermine) followed by exhaustive protection of the remaining amino groups. After purification by silica gel chromatography the distal carboxylic acid is deprotected and reacted with long chain hydrocarbon containing amines or alcohols to give amides or esters respectively. Such polyamine analogs can be represented by the following structure



n = 1 Aspartic acid
n = 2 Glutamic acid
X = N or O

wherein n can also be greater than 2, preferably up to about 10 (including 3, 4, 5, 6, 7, 8 and 9) and R is defined as provided for R_1 and R_2 in formula II above. The alpha amino group of the distal carboxylic acid containing amino acid may also be derivatized as described above in Formula II. Such compounds may be described as "inverted" amide or ester derivatives of the compounds described in Figure 2.

Similar hydrophobic PAs can be prepared by the use of cysteine, serine, or homo serine to link the hydrophobic and polyamine moieties indirectly. The hydrophobic PAs may also be linked via an ester linkage (like that possible via serine), a thioester linkage (like that possible via cysteine), a urea linkage (-N-CO-N-), a carbamate linkage (-O-CO-N- or -N-CO-O-), or an extended sulfonamide linkage (-NH-SO_2-),

As shown in Figure 1, the active ester is added to an excess of polyamine to produce a mixture of substituted and unsubstituted acyl polyamines. The remaining free amino groups of the polyamines can then be protected, such as via their ^{t}Boc or Cbz carbamates, and the desired orthogonally-protected products can be isolated. Full protection of the amino groups produces a more lipophilic product mixture which facilitates purification of the desired compound. The exemplary reaction scheme in Figure 1 results in two synthetic intermediates, one with 4 Boc and 1 Cbz carbamates and the other with 4 Cbz and 1 Boc carbamates. These intermediates allow the exposure of selectively either the distal or proximal (relative to the starting spermine polyamine) amino groups to be selectively deprotected by catalytic hydrogenation (see left branch of scheme) or acid treatment (see right branch of scheme), respectively. When viewed relative to the lysine

moiety, the distal and proximal amino groups may be considered the ε- or α- amino positions, respectively.

The deprotected amino groups may then be further modified via conventional amide chemistry. For example, and without limiting the invention, the deprotected amino groups may be acylated or alkylated with either an acyl chloride or sulfonyl chloride to produce PAs shown in Figure 2 as Series I and II, respectively. The positions may also be carboxylic acid activated with standard peptide coupling reagents such as DCC, PyPOP or HBTU (to produce Series III PAs) or aldehydes using reductive amination conditions (to produce Series IV PAs). Additional analogs are produced by reductive amination of the free amino precursor with one of the ketone reagents shown in Series V. Series VI analogs are produced via the reductive amination reaction using a free mono- or di-amine precursor and an excess of the carbonyl containing reagent shown in the Series VI portion of Figure 2. An alternative method to produce these di-substituted tertiary amine-containing molecules is the conjugate addition of the selectively protected amine precursor to an α, β-unsaturated carbonyl compound or an α, β-unsaturated nitrile compound.

The above described synthetic schemes may be conducted in a parallel fashion to permit the simultaneous production of multiple PAs. For example, the reaction scheme shown in Figure 1 may be started with a mixture of L- and D- forms of Boc-Lys-(Cbz)-ONP and spermine. This results in a possible 4 different amino groups (two based on each of the L- and D- forms, and two based on each of the distal and proximal amino groups) deprotection and subsequent modification. There are also two additional possible modifications where both amino groups are simultaneously deprotected for subsequent modification. This results in a total of 6 possible routes for modification.

Parallel acylation with just two acyl chlorides, such as by solution phase methods, would produce twelve different PAs. Each individual PA may then be purified and the protective groups on the polyamine portion removed before further characterization and use.

The invention also provides compositions containing one or more PAs, as well as acceptable salts thereof, in combination with an excipient, diluent or vehicle to facilitate its use or administration to a subject. Preferably, the compositions are formulated for pharmaceutical, therapeutic or agricultural uses. Pharmaceutically acceptable salts of the invention (which contain basic groups) are formed where appropriate with strong or

moderately strong, non-toxic, organic or inorganic acids in the presence of the basic amine by methods known in the art. Exemplary salts include, but are not limited to, maleate, fumarate, lactate, oxalate, methanesulfonate, ethanesulfonate, benzenesulfonate, tartrate, citrate, hydrochloride, hydrobromide, sulfate, phosphate and nitrate salts.

As stated above, the PAs of the invention possess the ability to inhibit polyamine transport, a property that is exploited in the treatment of any of a number of diseases or conditions, most notably cancer. A composition of this invention may be active *per se*, or may act as a "pro-drug" that is converted *in vivo* to active form.

The PAs of the invention, as well as the pharmaceutically acceptable salts thereof, may be incorporated into convenient dosage forms, such as capsules, impregnated wafers, tablets or injectable preparations. Solid or liquid pharmaceutically acceptable carriers may also be employed. Pharmaceutical compositions designed for timed or delayed release may also be formulated.

Optionally, the compositions contain anti-oxidants, surfactants and/or glycerides. Examples of anti-oxidants include, but not limited to, BHT, vitamin E and/or C. Examples of glycerides include, but are not limited to, one or more selected from acetylated or unsubstituted monoglycerides; medium chain triglycerides, such as those found in oils; and caprylocaproyl macrogol-8 glycerides.

Preferably, the compounds of the invention are administered systemically, *e.g.*, by injection or oral administration. When used, injection may be by any known route, preferably intravenous, subcutaneous, intramuscular, intracranial or intraperitoneal. Injectables can be prepared in conventional forms, either as solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

Solid carriers include starch, lactose, calcium sulfate dihydrate, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate and stearic acid. Liquid carriers include syrup, peanut oil, olive oil, saline, water, dextrose, glycerol and the like. Similarly, the carrier or diluent may include any prolonged release material, such as glyceryl monostearate or glyceryl distearate, alone or with a wax. When a liquid carrier is used, the preparation may be in the form of a syrup, elixir, emulsion, soft gelatin capsule, liquid containing capsule, sterile injectable liquid (e.g., a solution), such as an ampule, or an aqueous or nonaqueous liquid suspension. A summary of such pharmaceutical

compositions may be found, for example, in *Remington's Pharmaceutical Sciences,* Mack Publishing Company, Easton Pennsylvania (Gennaro 18th ed. 1990).

The pharmaceutical preparations are made following conventional techniques of pharmaceutical chemistry involving such steps as mixing, granulating and compressing, when necessary for tablet forms, or mixing, filling and dissolving the ingredients, as appropriate, to give the desired products for oral or parenteral administration. Other preparations for topical, transdermal, intravaginal, intranasal, intrabronchial, intracranial, intraocular, intraaural and rectal administration may also be prepared. The pharmaceutical compositions may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and so forth.

Although the preferred routes of administration are systemic, the pharmaceutical composition may be administered topically or transdermally, e.g., as an ointment, cream or gel; orally; rectally; *e.g.*, as a suppository, parenterally, by injection or continuously by infusion; intravaginally; intranasally; intrabronchially; intracranially; intraaurally; or intraocularly.

Intraaural formulations are particularly preferred for the treatment or alleviation of hearing loss due to chemotherapy.

For topical application, the compound may be incorporated into topically applied vehicles such as a salve or ointment. The carrier for the active ingredient may be either in sprayable or nonsprayable form. Non-sprayable forms can be semi-solid or solid forms comprising a carrier indigenous to topical application and having a dynamic viscosity preferably greater than that of water. Suitable formulations include, but are not limited to, solution, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like. If desired, these may be sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers, or salts for influencing osmotic pressure and the like. Preferred vehicles for non-sprayable topical preparations include ointment bases, e.g., polyethylene glycol-1000 (PEG-1000); conventional creams; gels; as well as petroleum jelly and the like.

Topical preparations are particularly preferred for the application of the present invention to the control of unwanted hair growth on skin.

Also suitable for topical application are sprayable aerosol preparations wherein the compound, preferably in combination with a solid or liquid inert carrier material, is packaged in a squeeze bottle or in admixture with a pressurized volatile, normally gaseous propellant. The aerosol preparations can contain solvents, buffers, surfactants, perfumes, and/or antioxidants in addition to the compounds of the invention.

For the preferred topical applications, especially for humans, it is preferred to administer an effective amount of the compound to a target area, *e.g.*, skin surface, mucous membrane, eyes, *etc.* This amount will generally range from about 0.001 mg to about 1 g per application, depending upon the area to be treated, the severity of the symptoms, and the nature of the topical vehicle employed.

The compositions of the invention may be administered alone or in combination with one or more additional compounds that are used to treat the disease or condition. For treating cancer, the PAs are given in combination with anti-tumor agents, such as mitotic inhibitors, *e.g.*, vinblastine; alkylating agents, *e.g.*, cyclophosphamide; folate inhibitors, *e.g.*, methotrexate, pritrexim or trimetrexate; antimetabolites, *e.g.*, 5-fluorouracil and cytosine arabinoside; intercalating antibiotics, *e.g.*, adriamycin and bleomycin; enzymes or enzyme inhibitors, *e.g.*, asparaginase; topoisomerase inhibitors, *e.g.*, etoposide; or biological response modifiers, *e.g.*, interferon and interleukin-2. In fact, pharmaceutical compositions comprising any known cancer therapeutic in combination with the PAs disclosed herein are within the scope of this invention. Such combinations may be utilized either by combining the components into a single composition for administration or by administering the components separately as part of one therapeutic protocol.

Most preferably, the present compounds are administered in combination with one or more polyamine synthesis inhibitors such as, but not limited to, inhibitors of ornithine decarboxylase such as DFMO, aceylenic putrescine, 1-aminooxy-3-aminopropane, antizyme, 2-butylputrescine, cadaverine, L-canaline, 5'-deoxy-5'-[N-methyl-N-[3-(aminooxy)ethyl]amino]adenosine, 5'-deoxy-5'-[N-methyl-N-[3-(hydrazinopropyl)amino]adenosine, diaminopropane, 1,3-diamino-2-propanol, 2-difluoromethyl putrescine, difluorophenylethyl(4-aminopropylamidinohydrazone), 2,3-dimethylputrescine, N-dimethylputrescine, 2-ethylputrescine, (+ or -)-alpha-fluoromethylornithine, 2-fluoro methylputrescine, 2-hexylputrescine, 2-hydrazinoornithine, ibuprofen, D-methyl acetylenic putrescine, methylglyoxal bis(3-aminopropylamininohydrazone), 2-methylornithine, 2-methylputrescine, 2-

monofluoromethyl-trans-dehydroornithine, 2-monofluoromethyl dehydroputrescine, monofluoromethylornithine, 2-monofluoromethyl putrescine, neomycin, D-ornithine, 2-pentylputrescine, p-phenylenediamine, phosphopeptide MG 25000, phosphothreonine, phosphotyrosine, 2-propylputrescine, putrescine, allo-S-adenosyl-L-methionine, S-ethylthioadenosine, methylthioadenosine, and 5'-methyl-thioadenosine as discussed in Zollner H. (1993) Handbook of Enzyme Inhibitors, 2nd Ed. Weinheim:Basel(Switzerland); inhibitors of S-adenosylmethionine decarboxylase, such as SAM486A (4-aminoindanon-1-(2'amidino)hydrazone dihydrochloride monohydrate), S-adenosyl-1,8-diamino-3-thiooctane, S-(5'-adenosyl)methylthio-2-aminooxyethan, S-adenosyl-3-methylthio-1-propylamine, 5'-{[(Z)-4-amino-2-butenyl]methylamino}-5'-deoxyadenosine, 5'-amino-5'-deoxyadenosine, 5'-[(aminoiminomethyl)amino]-5']deoxyadenosine dihydrogensulphate, 1-aminooxy-3-aminopropane, [2-(aminooxy)ethyl](5'-deoxyadenosine-5'-yl)(methyl)sulphonium, 5'-[(3-aminopropyl]-amino)-5'-deoxyadenosine, 5'-[(3-aminopropyl]-nethylamino)-5'-deoxyadenosine, 9-[6(RS)-amino-5,6,7-trideoxy-beta-D-ribo-octofuranosyl]-9H-purin-6-amine, borohydride, n-butylglyoxal bis(guanylhydrazone), 9-[6(RS)-c-carboxamido-5,6,7-trideoxy-beta-D-ribo-octofuranosyl]-9H-purin-6-amine, cyanide, cyanoborohydride, S-(5'deoxy-5'adenosyl)methionylethylhydroxylamine, S-(5'deoxy-5'adenosyl)methionylthiohydroxylamine, 5'-deoxy-5'-[N-methyl-N-[2-(aminooxy)ethyl]amino]adenosine, 9-[6(S)-diamino-5,6,7,8,9-pentadeoxy-beta-D-ribo-nanofuranosyl]-9H-purin-6-amine, diethylglyoxal bis(guanylhydrazone), difluorophynylethyl (4-aminopropylamidinohydrazone), dimethyl(5'-adenosyl)sulfonium, dimethylglyoxal bis(guanylhydrazone), ethylglyoxal bis(guanylhydrazone), hydroxylamine, 4-hydroxypenenal, MDL 73811, 5'[[3-methylamino)propyl]amino]-5'-deoxyadenosine(1,1'-(methylethanediylidine)dinitro)bis(3aminoguanididne), methylglyoxal bis(3-aminopropylamidinohydrazone), methylglyoxal bis(cyclohexylamidinohydrazone), methylglyoxal bis(guanylhydrazone), pentanedialdehyde bis guanylhydrazone), phenylhydrazine, propanedialdehyde bis(guanylhydrazone), semicarbazide, sodium borohydride, sodium cyanoborohydride, and spermine as discussed in Zollner H. (1993) Handbook of Enzyme Inhibitors, 2nd Ed.

The PAs of the invention may also be used in combination with monoclonal antibodies and tumor vaccines as well as with cellular therapy in subjects undergoing treatment for human diseases such as cancer. The PAs may also be used for chemoprevention in subjects at risk for developing cancer wherein one or more PAs are

taken alone or in combination with a polyamine synthesis inhibitor to prevent the onset or recurrence of cancer.

The pharmaceutical compositions of the invention may also comprise one or more other medicaments such as anti-infectives including antibacterial, anti-fungal, anti-parasitic, anti-viral, and anti-coccidial agents.

Typical single dosages of the compounds of this invention are between about 1 ng and about 10 g/kg body weight. The dose is preferably between about 0.01 mg and about 1g/kg body wt. and, most preferably, between about 0.1 mg and about 100 mg/kg body wt. For topical administration, dosages in the range of about 0.01-20% concentration of the compound, preferably 1-5%, are suggested. A total daily dosage in the range of about 1-500 mg is preferred for oral administration. The foregoing ranges are, however, suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are expected and may be routinely made by those skilled in the art.

Effective amounts or doses of the compound for treating a disease or condition can be determined using recognized *in vitro* systems or *in vivo* animal models for the particular disease or condition. In the case of cancer, many art-recognized models are known and are representative of a broad spectrum of human tumors. The compounds may be tested for inhibition of tumor cell growth in culture using standard assays with any of a multitude of tumor cell lines of human or nonhuman animal origin. Many of these approaches, including animal models, are described in detail in Geran, R.I. *et al.*, "Protocols for Screening Chemical Agents and Natural Products Against Animal Tumors and Other Biological Systems (Third Edition)", *Canc. Chemother. Reports*, Part 3, 3:1-112.

The present invention also provides methods of using the PAs, whether formulated in compositions or not, to inhibit cell growth and proliferation when used alone or in combination with a polyamine synthesis inhibitor. Such methods may be readily conducted by systemic or local administration of the PAs. Local delivery of a PA provides a high local concentration while reducing the likelihood of systemic effects on polyamine metabolism that may result from systemic PA administration.

The inhibition of cellular growth and proliferation is advantageously conducted with the contemporaneous administration of one or more inhibitors of polyamine synthesis. Such inhibition may be applied toward a variety of cell types, including, but not limited to,

bacterial cells, fungal cells, and the eukaryotic cells of higher multicellular organisms. In one application of the invention, one or more PAs may be used to inhibit bacterial or fungal cell growth. This embodiment may be advantageously used in both the clinic and agriculture to control bacteria or fungi.

In another embodiment of the invention, one or more PAs may be used in combination with an inhibitor of polyamine synthesis to inhibit the growth and/or proliferation of cancer cells, including those of solid tumors. While this latter application may be performed in any multicellular organism, most preferred are applications of the invention for use in human subjects.

Additionally, the invention provides for the use of one or more PAs for analytical and/or preparative methods relating to polyamine transport. For example, and without limiting the invention, a PA may be used to identify and/or localize a polyamine transporter by virtue of physical binding between the PA and the transporter and the presence of a label linked to the PA. Suitable labels are well known in the art, and they permit the identification or localization of the PA either because the label itself emits a detectable signal, or by virtue of its affinity for a label-specific partner which is detectable or becomes so by binding to, or otherwise reacting with, the label. Examples of labels include, but are not limited to, radioactive isotopes, fluorescent tags, and proteinaceous tags. The methods of identification and /or localization provided by the invention may be used in whole or as part of a diagnostic or research protocol.

The invention also provides preparative uses of the PAs. For example, one or more PAs can be used to bind and isolate proteins or other cellular factors that interact with polyamines. An exemplar of such a method is the use of a PA to bind to a polyamine transporter and permit its isolation or purification. These methods can be performed in solution, where interaction between a PA and a PA binding protein or factor results in a complex that may be subsequently isolated or purified from solution, or in solid phase, where a PA is immobilized and interactions between the PA and a PA binding protein or factor results in a complex of the protein or factor with the immobilized PA.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLE I

Chemical Synthesis of Polyamine Agents (PAs)

PAs analogs were synthesized in a parallel fashion starting from the orthogonally protected diamino containing amino acid starting materials. The use of the 4-nitrophenyl activated ester *L*-Boc-Lys-(Cbz)-ONP in Figure 1 provides an exemplary illustration of the synthetic process. The active ester is added dropwise to a solution of 1.5 equivalents of polyamine in methanol to give a statistical mixture of unsubstituted, mono-substituted and di-substituted acyl polyamines. Following evaporation of the solvent, the remaining free amino groups in the polyamine moiety are protected either as their ^{t}Boc or Cbz carbamates. Standard workup results in a completely protected crude product mixture. The desired orthogonally-protected product is isolated in pure form by silica gel chromatography using standard organic solvents. This purification process is based on separation of polyamine molecules with the remaining amino groups being fully protected, which provides a much more lipophilic product mixture that greatly facilitates the purification process. Thus the exemplary intermediates containing either 4 Boc groups or 4 Cbz groups in addition to the acyl functionality remained lipophilic enough to purify using standard solvents including a one to one mixture of ethyl acetate and hexanes containing various proportions of methanol (0 to 10%).

As shown in Figure 1, the approach provides two synthetic intermediates, one with 4 Boc and 1 Cbz carbamates and the other with 4 Cbz and 1 Boc carbamates. These intermediates allow the exposure of only one amino group, either the proximal (α–) or distal (ε-), in a selective manner. It is also possible to modify this approach such that both amino groups are exposed for further modification. The selective deprotection of either the proximal (α–) or distal (ε-) amino group as shown in Figure 1 may occur via catalytic hydrogenation or acid treatment, respectively. The exposed amino groups were then acylated or alkylated with either an acyl chloride or sulfonyl chloride to produce Series I and II (see Figure 2) type PAs, respectively. The exposed amino groups may also be carboxylic acid activated with standard peptide coupling reagents such as DCC, PyPOP or HBTU (to produce Series III type PAs) or aldehydes under reductive amination conditions (to produce Series IV type PAs). Additional analogs are produced by reductive amination of the free amino precursor with one of the ketone reagents shown in Series V. Series VI

analogs are produced via the reductive amination reaction using a free mono- or di-amine precursor and an excess of the carbonyl reagent that are shown in the Series VI chart. An alternative method to produce these di-substituted tertiary amine-containing molecules is the conjugate addition of the selectively protected amine precursor to an α, β-unsaturated carbonyl compound or an α, β-unsaturated nitrile compound.

Deprotections of isolated PAs using standard conditions gave the desired products in pure form. The PAs were characterized by thin layer chromatography (TLC) analysis (using ^{i}PrOH/HOAc/pyr/H_2O, 4:1:1:2); high performance liquid chromatography (HPLC) analysis (dansylation followed by HPLC using fluorescent detection); liquid chromatography-mass spectroscopy (LC-MS) by electrospray ionization; and ^{1}H and ^{13}C NMR analysis. All PAs were estimated to be 90 to 98% pure following synthesis.

EXAMPLE II

Cell Culture and Reagents

All cell lines were obtained from ATCC (Manassas, VA) and cultured in the recommended media, serum, and CO_2 concentration. Medias were obtained from Mediatech, Inc. (Herndon, VA) and serums from Gibco BRL (Gaithersburg, MD). 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mM L-glutamine (all from BioWhittaker, Walkersville, MD) were included in all cultures. DFMO was obtained from Marion Merrell Dow (Cinncinati, OH). When cells were cultured with polyamines or ORI compounds, 1 mM aminoguanidine (AG; Sigma) was included to inhibit serum amine oxidase activity. IC_{50} refers to the concentration of PA that results in 50% of maximum cell growth inhibition in the presence of PA alone.

EXAMPLE III

Polyamine Transport and Ki Assays

[2,9-^{3}H]spermidine (SPD) from DuPont NEN, Boston, MA was added alone or simultaneously with PAs to 24-well plates containing MDA-MB-231 cells in log growth. The cells were incubated at 37°C for 15 min to determine initial rate polyamine uptake. The cells were then washed three times with cold PBS, lysed with 0.1% SDS, and the amount of polyamine incorporation into the cells was determined by scintillation counting of the cell lysates. To determine a K_i, four radioactive substrate concentrations (0.3-3 μM)

and five inhibitor concentrations (0.01-1.0 μM) and a control were tested. The K_i values were determined using double reciprocal Lineweaver-Burke plot analyses. K_i values were determined from linear equations derived from graphing the slopes of Lineweaver-Burke plot vs. inhibitor concentration, with K_i = y-intercept / slope. Results of these analyses are shown in Table 3 above.

EXAMPLE IV

Growth Inhibition Assay

Cells were plated in 96-well plates such that they would be in log growth for the duration of the assay. The day after plating, PAs were added to the cells, and growth, if any, permitted to continue for six days in the presence of 1 mM AG and 0.5 μM SPD to insure that any growth inhibition was not the result of depletion of external polyamines in the media. At the end of the six days, cell growth was measured by MTS/PMS dye assay (Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay; Promega, Madison, WI). EC_{50} represents the concentration of PA that resulted in 50% of maximum growth inhibition achievable in the presence of both DFMO (5 mM in all cell lines except MDA) and PA (at different concentrations depending in part on the cell line used) compared to controls. IC_{50} represents the concentration of PA that resulted in 50% maximum growth inhibition when used alone. Results are shown in Table 3 above.

EXAMPLE V

HPLC Analysis of Dansylated Derivatives

Sample handling for Polyamine Analysis (see Kabra, Pokar M., Hsian K. Lee, Warren P Lubich and Laurence J. Marton: Solid-Phase Extraction and Determination of Dansyl Derivatives of Unconjugated and Acetylated Polyamines by Reverse-Phase Liquid Chromatography: Improved Separation Systems for Polyamines in Cerebrospinal Fluid, Urine and Tissue. Journal of Chromatography 380 (1986) 19-32)

Plasma samples (from blood)- remove 125-150μl sample (optimally) into a microfuge tube and mix 1:1 with 0.4M perchloric acid. Vortex and spin down sample at 13000rpm for 10 minutes in 5°C centrifuge. Remove 200μl supernatant for dansylation as described in

dansylation protocol. Plasma samples as small as 25µl may be analyzed (for this and the following discussion, any sample that does not yield 200µl supernatant for dansylation may have its volume increased to 200µl with perchloric acid for the dansylation protocol).

Cell culture samples

Media- remove 1.5ml into 1.7ml microfuge tube and spin at 3000rpm for 5minutes in 5°C centrifuge. Remove 300µl supernatant and mix 1:1 with cold 0.4M perchloric acid. Vortex and spin down sample at 13000rpm for 10minutes in 5°C centrifuge. Remove 200µl supernatant for dansylation as described in dansylation protocol.

Cells- Trypsinize as usual and spin in 15ml tube 6 min at 4° at 1500 rpm. Pour off supernatant and resuspend pellet in 1.5ml 1X PBS. Transfer to large microfuge tube. Spin at 3000rpm at 4° for 5 minutes. Remove supernatant. Resuspend pellet in 1.0ml 1X PBS. Remove 20µl for counting and spin @ 3000rpm @4° for 5minutes. Remove supernatant. To the dry pellet, add 200µl 0.4M perchloric acid per 10^6 cells. Pipette up and down to mix. Vortex and spin down sample at 13000rpm for 10minutes in 5°C centrifuge. Remove 200µl supernatant for dansylation as described in dansylation protocol. Remainder of supernatant can be stored at -70°C.

Tissues- Keep samples on ice during preparation. Cut an approximately 100mg piece from tissue sample and place into 15ml conical tube. Add 1.2M perchloric acid in a 20:1 vol/weight ratio (i.e. 2ml/100mg). Homogenize tissue using a tissue grinder. Vortex sample and remove 1ml into a microfuge tube. Spin at 13000 rpm for 10 minutes in 5°C centrifuge. Remove 200µl supernatant for dansylation as described in dansylation protocol.

Dansylation Protocol for Polyamine Analysis

200µl sample in Perchloric acid

10µl Internal Standard (IS) (1,7-diaminoheptane, 100µM stock); use 20µl for 25min and 1483 HPLC

120µl saturated sodium carbonate solution (360µl is used for tissue samples)

400µl dansyl chloride solution (made fresh, 10mg/ml in acetone)

Add all ingredients to a 4ml screw cap glass vial and vortex for 30 seconds. Float vials in 70°C water bath for 10 minutes. Remove and allow cooling to room temp in dark, as samples are light sensitive. Proceed to sample prep protocol once samples have cooled.

Sample Prep Protocol

Alltech C-18 maxi-prep cartridges are used, one for each sample dansylated, to clean any interfering reactions from the samples. This process also places the samples in methanol for application to the HPLC system.

Each cartridge is placed on a vacuum manifold and washed once with 3ml MeOH followed by 3ml H_2O. Samples are then removed by 1ml syringe from the glass vials and applied to the Alltech cartridges. Each cartridge is then washed with 10ml H_2O and dried 2x with 30cc syringe of air.

All steps to this point are allowed discarded. The cartridges are placed with a tube rack with labeled 1.7ml microfuge tubes for elution. Samples are eluted with 1ml MeOH into the microfuge tubes. Samples are now ready for injection onto HPLC or can be stored at –70°C for up to several months if necessary.

The solvents used in the above are as follows:

Solvent A: HPLC grade Acetonitrile

Solvent B: 10mM Na acetate pH 4.5/ 10% acetonitrile (8.9L H_2O, 1L Acetonitrile, 100ml 1M Na acetate pH 4.5, mix well, filter and store at room temp).

Sample Injection: loop overfill is achieved by injecting 100µl onto a 20µl loop. Samples are kept at 4°C until injection by a water cooled storage rack on the 231XL auto injector.

40 minute PA analysis:

Gradient:	time	%A	%B
	0	48	52
	25	90	10
	30	100	0
	35	48	52

40 48 52

Flow rate is 3 ml/minute

Solutions and Sources are as follows:

Internal Standard: 1,7-Diaminoheptane (Sigma D-3266)

Made up 20mM in H_2O, and stored at –70°C. Diluted to 100μM working stock in H_2O and also stored at –70°C.

Perchloric acid: 70% ACS reagent (Aldrich 244252)

For 0.4M, mix 3.4ml in a total of 100ml H_2O. Store at room temp.

For 1.2M, mix 10.2ml in a total of 100ml H_2O. Store at room temp.

Sodium carbonate: anhydrous (Acros 42428-5000)

Make a saturated solution in H_2O.

Sodium acetate: anhydrous (Sigma S-2889)

Make up 1M in H_2O, then pH to 4.5 with glacial acetic acid. Filter and store at room temp.

Dansyl chloride: 95% (Sigma D-2625)

Acetonitrile: HPLC grade (Fisher A998-4)

Methanol: HPLC grade (Fisher A452-4)

Acetone: HPLC grade (Fisher A949-1)

Glacial acetic acid: ACS reagent (Fisher A38212)

All references cited herein, including patents, patent applications, and publications, are hereby incorporated by reference in their entireties, whether previously specifically incorporated or not. As used herein, the terms "a", "an", and "any" are each intended to include both the singular and plural forms.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation. While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such

departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

Claims

1. A polyamine analog or derivative represented by the formula

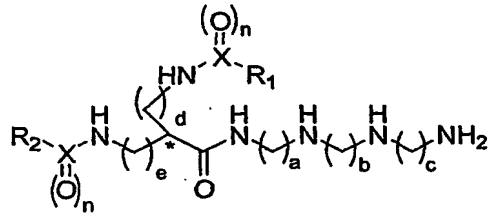
R-X-L-polyamine

wherein R is a straight or branched C10-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy; a C1-8 alicyclic; a single or multiring aryl substituted or unsubstituted aliphatic; an aliphatic-substituted or unsubstituted single or multiring aromatic; a single or multiring heterocyclic; a single or multiring heterocyclic aliphatic; an aryl sulfonyl;

X is -CO-, $-SO_2-$, or $-CH_2-$; and

L is a covalent bond or a naturally occurring amino acid, ornithine, 2,4-diaminobutyric acid, or derivatives thereof.

2. A polyamine analog or derivative represented by formula II:



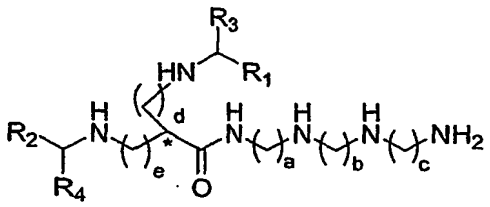
wherein a, b, and c independently range from 1 to 10; d and e independently range from 0 to 30; each X is independently either a carbon (C) or sulfur (S) atom, and R_1 and R_2 are independently selected from H or from the group of a straight or branched C1-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy; a C1-8 alicyclic; a single or multiring aryl substituted or unsubstituted aliphatic; an aliphatic-substituted or unsubstituted single or multiring aromatic; a single or multiring heterocyclic; a single or multiring heterocyclic aliphatic; a C1-10 alkyl; an aryl sulfonyl; or cyano; or

each of $R_1X\{O\}_n-$ and $R_2X\{O\}_n-$ are independently replaced by H;

wherein * denotes a chiral carbon position; and

wherein if X is C, then n is 1; if X is S, then n is 2; and if X is C, then the XO group may be CH_2 such that n is 0.

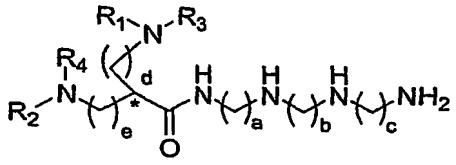
3. A polyamine analog or derivative represented by formula III:



wherein a, b, and c independently range from 1 to 10 and d and e independently range from 0 to 30; and

R_1, R_2, R_3, and R_4 may be the same or different and are independently selected from H or from the group of a straight or branched C1-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy; a C1-8 alicyclic; a single or multiring aryl substituted or unsubstituted aliphatic; an aliphatic-substituted or unsubstituted single or multiring aromatic; a single or multiring heterocyclic; a single or multiring heterocyclic aliphatic; a C1-10 alkyl; an aryl sulfonyl; or cyano.

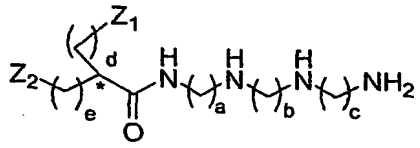
4. A polyamine analog or derivative represented by formula IV:



wherein a, b, and c independently range from 1 to 10 and d and e independently range from 0 to 30; and

R_1, R_2, R_3, and R_4 may be the same or different and are independently selected from H or from the group of a straight or branched C1-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy; a C1-8 alicyclic; a single or multiring aryl substituted or unsubstituted aliphatic; an aliphatic-substituted or unsubstituted single or multiring aromatic; a single or multiring heterocyclic; a single or multiring heterocyclic aliphatic; a C1-10 alkyl; an aryl sulfonyl; or cyano.

5. A polyamine analog or derivative represented by formula V:



wherein a, b, and c independently range from 1 to 10 and d and e independently range from 0 to 30; and

wherein Z_1 is NR_1R_3 and Z_2 is selected from $-R_1$, $-CHR_1R_2$ or $-CR_1R_2R_3$ or Z_2 is NR_2R_4 and Z_1 is selected from $-R_1$, $-CHR_1R_2$ or $-CR_1R_2R_3$

wherein R_1, R_2, and R_3 may be the same or different and are independently selected from H or from the group of a straight or branched C1-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy; a C1-8 alicyclic; a single or multiring aryl substituted or unsubstituted aliphatic; an aliphatic-substituted or unsubstituted single or multiring aromatic; a single or multiring heterocyclic; a single or multiring heterocyclic aliphatic; a C1-10 alkyl; an aryl sulfonyl; or cyano.

6. The analog or derivative of any one of claims 1-5 wherein said a, b, and c are such that the analog or derivative is putrescine, spermine or spermidine based.

7. The analog or derivative of any one of claims 1-5 wherein each of R_1, R_2, R_3, and R_4 is independently selected from H or a straight or branched C10-50 saturated or unsaturated aliphatic, carboxyalkyl, carbalkoxyalkyl, or alkoxy.

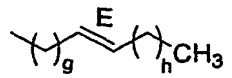
8. The analog or derivative of claim 1 wherein L is an amino acid selected from lysine, aspartic acid, glutamic acid, ornithine, or 2,4-diaminobutyric acid

9. A polyamine analog or derivative selected from spermine based compounds IA4, IB4, IA7, IVB22 or IVA22 as illustrated in Figure 2.

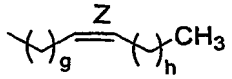
10. A polyamine analog or derivative selected from the compounds depicted in Figure 12.

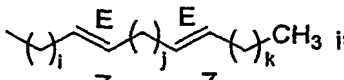
11. The analog or derivative of any one of claims 1-5 wherein d is 4 and e is 0.

12. The analog or derivative of any one of claims 1-5 wherein each of R_1, R_2, R_3, and R_4 is independently selected from H or from

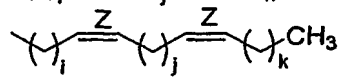


g=0-15, h=0-15





i=0-15, j=0-15, k=0-15



wherein each of g, h, i, j, and k are independently selected from 0 to 15 and

wherein E refers to "entgegen" and Z refers to "zusammen".

13. A composition comprising a polyamine analog or derivative according to any one of claims 1-12 and an excipient, diluent or vehicle.

14. The composition of claim 13 wherein said excipient, diluent or vehicle is pharmaceutically or cosmetically acceptable.

15. The composition of claim 13 wherein said excipient, diluent or vehicle is for topical or intra-aural administration.

16. The composition of claim 13 further comprising a polyamine biosynthesis inhibitor.

17. The composition of claim 16 wherein said inhibitor is DFMO.

18. The composition of claim 13 formulated for intravenous, subcutaneous, intramuscular, intracranial, intraperitoneal, topical, transdermal, intravaginal, intranasal, intrabronchial, intracranial, intraocular, intraaural, rectal, or parenteral administration

19. A method of treating one or more conditions selected from cancer, osteoporosis, asthma, autoimmune diseases, rheumatoid arthritis, systemic lupus erythematosus, Type I insulin dependent diabetes, psoriasis, restenosis, inhibition of unwanted proliferation of hair on skin, tissue transplantation, African sleeping sickness, inflammation, hyperparathyroidism, treatment of peptic ulcer, glaucoma, Alzheimer's disease, suppression of atrial tachycardias, stimulation or inhibition of intestinal motility, Crohn's disease and other inflammatory bowel diseases, high blood pressure (vasodilation), stroke, epilepsy, anxiety, neurodegenerative diseases, hyperalgesic states, the protection of hair cells from chemotherapeutic-induced loss of hearing, and pharmacological manipulation of cocaine reinforcement and craving in treating cocaine addiction and overdose comprising administration of an analog or derivative of any one of claims 1-12 or a composition of any one of claims 13-18 to a subject afflicted with said one or more conditions.

20. The method of claim 19 wherein said administration is systemic.

21. The method of claim 19 or 20 wherein said administration is oral.

22. The method of claim 19 or 20 wherein said administration is via a time release vehicle.

23. A method of treating fungal, bacterial, viral, or parasitic diseases comprising administration of an analog or derivative of any one of claims 1-12 or a composition of any one of claims 13-18 to a subject afflicted with said disease.

24. A method of enhancing cellular uptake of nucleic acids comprising contacting a cell with an analog or derivative of any one of claims 1-12.

25. A method of inhibiting hair growth comprising topical administration of an analog or derivative of any one of claims 1-12 or a composition of any one of claims 13-18 to a subject in need of hair growth inhibition.

26. The method of claim 25 wherein said analog or derivative is formulated as a cosmetic.

27. A method of inhibiting hearing loss comprising administration of an analog or derivative of any one of claims 1-12 to a subject in need of said inhibition.

28. The method of claim 27 wherein said subject is susceptible to hearing loss due to cancer chemotherapy.

Figure 1. Scheme 1: Synthesis of selectively acylated Lys-Spm conjugates.

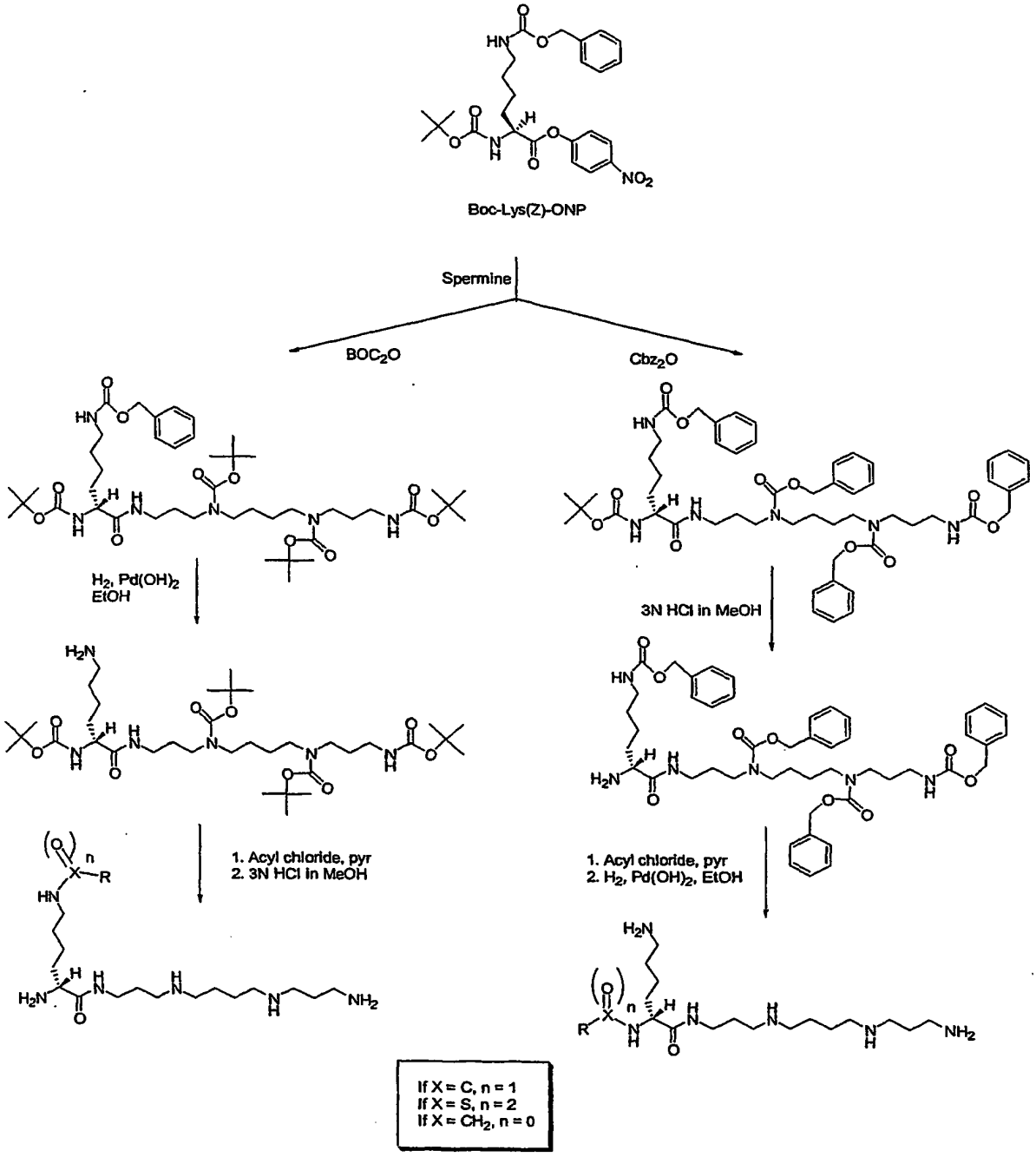
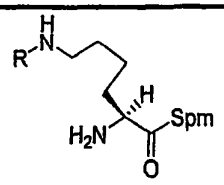
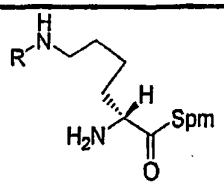
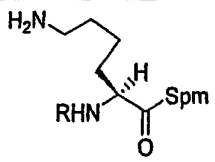
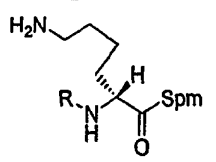
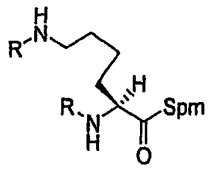
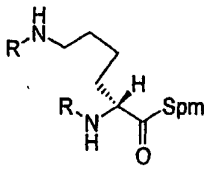
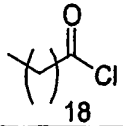
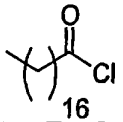
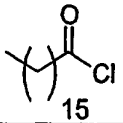
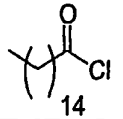
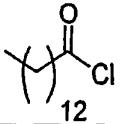
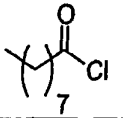
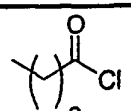
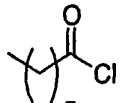
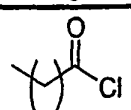
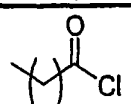
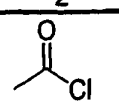
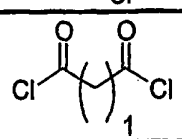
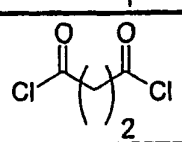
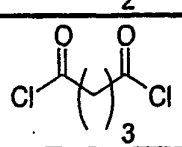
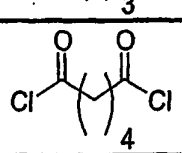
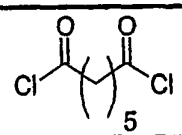
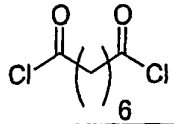
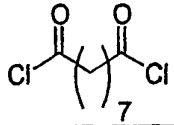
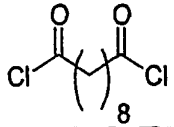
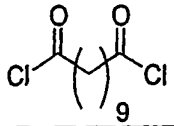
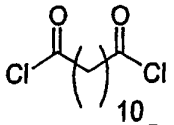
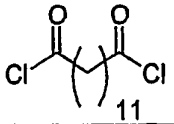
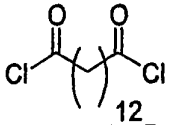
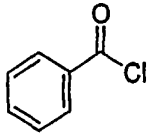
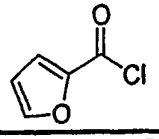
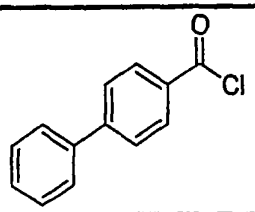
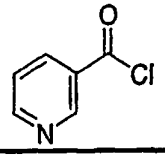
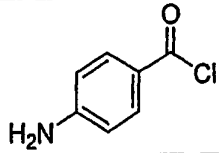
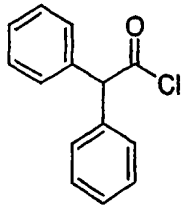
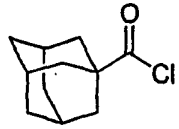


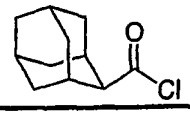
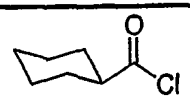
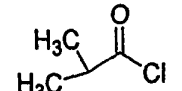
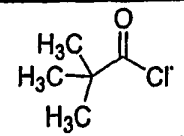
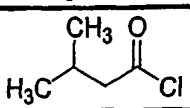
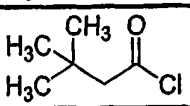
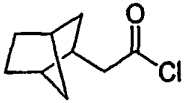
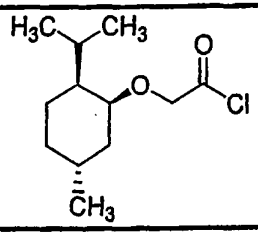
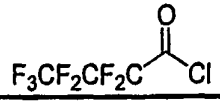
Figure 2

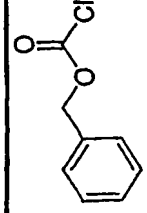
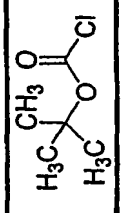
		A	B	C	D	E	F
	SERIES I	 A (ε-*L*-Lys)	 B (ε-*D*-Lys)	 C (α-*L*-Lys)	 D (α-*L*-Lys)	 E (α,ε-*L*-Lys)	 F (α, ε-*D*-Lys)
1		IA1	IB1	IC1	ID1	IE1	IF1
2		IA2	IB2	IC2	ID2	IE2	IF2
3		IA3	IB3	IC3	ID3	IE3	IF3
4		IA4	IB4	IC4	ID4	IE4	IF4
5		IA5	IB5	IC5	ID5	IE5	IF5
6		IA6	IB6	IC6	ID6	IE6	IF6

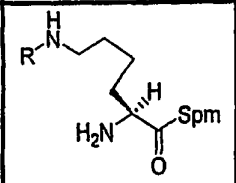
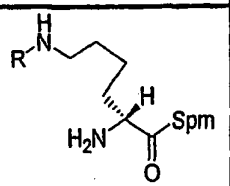
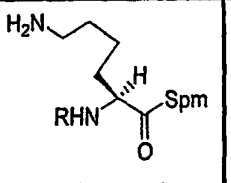
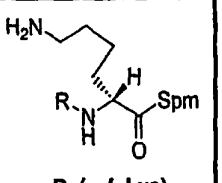
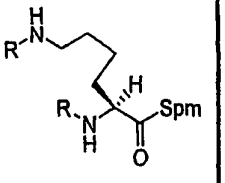
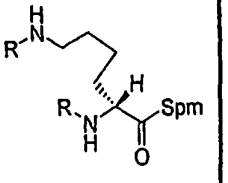
7		IA7	IB7	IC7	ID7	IE7	IF7
8		IA8	IB8	IC8	ID8	IE8	IF8
9		IA9	IB9	IC9	ID9	IE9	IF9
10		IA10	IB10	IC10	ID10	IE10	IF10
11		IA11	IB11	IC11	ID11	IE11	IF11
12		IA12	IB12	IC12	ID12	IE12	IF12
13		IA13	IB13	IC13	ID13	IE13	IF13
14		IA14	IB14	IC14	ID14	IE14	IF14
15		IA15	IB15	IC15	ID15	IE15	IF15

16		IA16	IB16	IC16	ID16	IE16	IF16
17		IA17	IB17	IC17	ID17	IE17	IF17
18		IA18	IB18	IC18	ID18	IE18	IF18
19		IA19	IB19	IC19	ID19	IE19	IF19
20		IA20	IB20	IC20	ID20	IE20	IF20
21		IA21	IB21	IC21	ID21	IE21	IF21
22		IA22	IB22	IC22	ID22	IE22	IF22
23		IA23	IB23	IC23	ID23	IE23	IF23

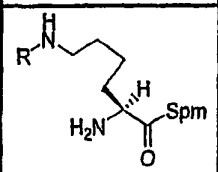
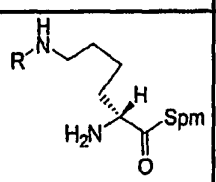
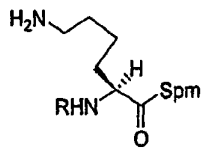
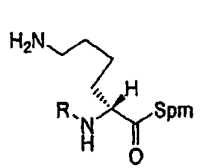
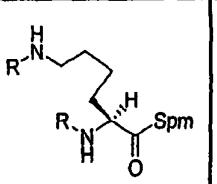
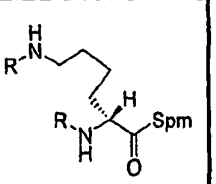
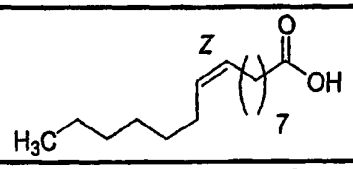
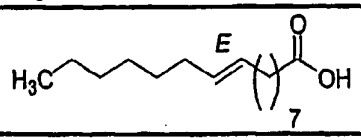
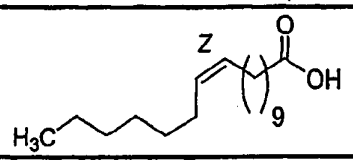
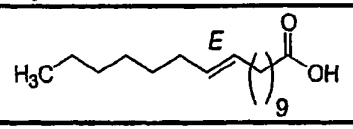
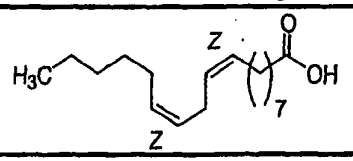
24		IA24	IB24	IC24	ID24	IE24	IF24
25		IA25	IB25	IC25	ID25	IE25	IF25
26		IA26	IB26	IC26	ID26	IE26	IF26
27		IA27	IB27	IC27	ID27	IE27	IF27
28		IA28	IB28	IC28	ID28	IE28	IF28
29		IA29	IB29	IC29	ID29	IE29	IF29
30		IA30	IB30	IC30	ID30	IE30	IF30

31		IA31	IB31	IC31	ID31	IE31	IF31
32		IA32	IB32	IC32	ID32	IE32	IF32
33		IA33	IB33	IC33	ID33	IE33	IF33
34		IA34	IB34	IC34	ID34	IE34	IF34
35		IA35	IB35	IC35	ID35	IE35	IF35
36		IA36	IB36	IC36	ID36	IE36	IF36
37		IA37	IB37	IC37	ID37	IE37	IF37
38		IA38	IB38	IC38	ID38	IE38	IF38
39		IA39	IB39	IC39	ID39	IE39	IF39

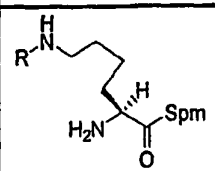
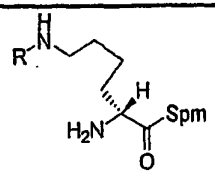
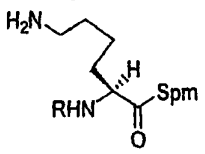
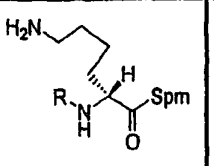
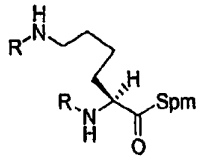
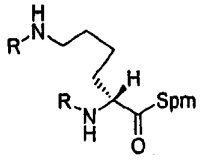
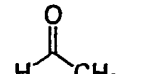
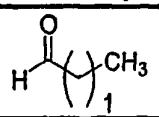
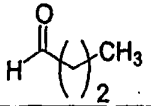
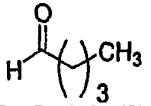
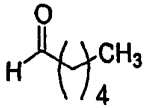
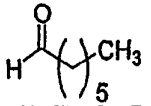
40		IA40	IB40	IC40	ID40	IE40	IF40
41		IA41	IB41	IC41	ID41	IE41	IF41

		A	B	C	D	E	F
	SERIES II	 A (ε-*L*-Lys)	 B (ε-*D*-Lys)	 C (α-*L*-Lys)	 D (α-*L*-Lys)	 E (α,ε-*L*-Lys)	 F (α, ε-*D*-Lys)
1	CH_3SO_2Cl	**IIA1**	**IIB1**	**IIC1**	**IID1**	**IIE1**	**IIF1**
2	$CH_3CH_2SO_2Cl$	**IIA2**	**IIB2**	**IIC2**	**IID2**	**IIE2**	**IIF2**
3	$CH_3(CH_2)_2SO_2Cl$	**IIA3**	**IIB3**	**IIC3**	**IID3**	**IIE3**	**IIF3**
4	$CH_3(CH_2)_3SO_2Cl$	**IIA4**	**IIB4**	**IIC4**	**IID4**	**IIE4**	**IIF4**
5	$CH_3(CH_2)_6SO_2Cl$	**IIA5**	**IIB5**	**IIC5**	**IID5**	**IIE5**	**IIF5**
6	$CH_3(CH_2)_8SO_2Cl$	**IIA6**	**IIB6**	**IIC6**	**IID6**	**IIE6**	**IIF6**
7	$CH_3(CH_2)_{10}SO_2Cl$	**IIA7**	**IIB7**	**IIC7**	**IID7**	**IIE7**	**IIF7**
8	$CH_3(CH_2)_{12}SO_2Cl$	**IIA8**	**IIB8**	**IIC8**	**IID8**	**IIE8**	**IIF8**
9	$CH_3(CH_2)_{14}SO_2Cl$	**IIA9**	**IIB9**	**IIC9**	**IID9**	**IIE9**	**IIF9**
10	$CH_3(CH_2)_{15}SO_2Cl$	**IIA10**	**IIB10**	**IIC10**	**IID10**	**IIE10**	**IIF10**
11	$CH_3(CH_2)_{16}SO_2Cl$	**IIA11**	**IIB11**	**IIC11**	**IID11**	**IIE11**	**IIF11**
12	$CH_3(CH_2)_{17}SO_2Cl$	**IIA12**	**IIB12**	**IIC12**	**IID12**	**IIE12**	**IIF12**
13	$CH_3(CH_2)_{18}SO_2Cl$	**IIA13**	**IIB13**	**IIC13**	**IID13**	**IIE13**	**IIF13**
14	$CH_3(CH_2)_{19}SO_2Cl$	**IIA14**	**IIB14**	**IIC14**	**IID14**	**IIE14**	**IIF14**
15	$CH_3(CH_2)_{20}SO_2Cl$	**IIA15**	**IIB15**	**IIC15**	**IID15**	**IIE15**	**IIF15**

16		IIA16	IIB16	IIC16	IID16	IIE16	IIF16
17		IIA17	IIB17	IIC17	IID17	IIE17	IIF17
18		IIA18	IIB18	IIC18	IID18	IIE18	IIF18
19		IIA19	IIB19	IIC19	IID19	IIE19	IIF19
20		IIA20	IIB20	IIC20	IID20	IIE20	IIF20
21		IIA21	IIB21	IIC21	IID21	IIE21	IIF21

		A	B	C	D	E	F
	SERIES III	 A (ε-*L*-Lys)	 B (ε-*D*-Lys)	 C (α-*L*-Lys)	 D (α-*L*-Lys)	 E (α,ε-*L*-Lys)	 F (α, ε-*D*-Lys)
1		IIIA1	IIB1	IIIC1	IIID1	IIIE1	IIIF1
2		IIIA2	IIIB2	IIIC2	IIID2	IIIE2	IIIF2
3		IIIA3	IIIB3	IIIC3	IIID3	IIIE3	IIIF3
4		IIIA4	IIIB4	IIIC4	IIID4	IIIE4	IIIF4
5		IIIA5	IIIB5	IIIC5	IIID5	IIIE5	IIIF5

6	IIIA6	IIIB6	IIIC6	IIID6	IIIE6	IIIF6
7	IIIA7	IIIB7	IIIC7	IIID7	IIIE7	IIIF7

		A	B	C	D	E	F
	SERIES IV	 A (ε-*L*-Lys)	 B (ε-*D*-Lys)	 C (α-*L*-Lys)	 D (α-*L*-Lys)	 E (α,ε-*L*-Lys)	 F (α, ε-*D*-Lys)
1		IVA1	IVB1	IVC1	IVD1	IVE1	IVF1
2		IVA2	IVB2	IVC2	IVD2	IVE2	IVF2
3		IVA3	IVB3	IVC3	IVD3	IVE3	IVF3
4		IVA4	IVB4	IVC4	IVD4	IVE4	IVF4
5		IVA5	IVB5	IVC5	IVD5	IVE5	IVF5
6		IVA6	IVB6	IVC6	IVD6	IVE6	IVF6

7		IVA7	IVB7	IVC7	IVD7	IVE7	IVF7
8		IVA8	IVB8	IVC8	IVD8	IVE8	IVF8
9		IVA9	IVB9	IVC9	IVD9	IVE9	IVF9
10		IVA10	IVB10	IVC10	IVD10	IVE10	IVF10
11		IVA11	IVB11	IVC11	IVD11	IVE11	IVF11
12		IVA12	IVB12	IVC12	IVD12	IVE12	IVF12
13		IVA13	IVB13	IVC13	IVD13	IVE13	IVF13
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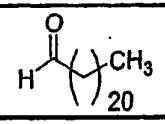
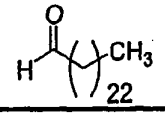
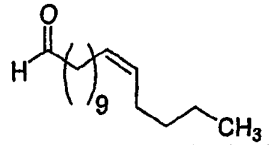
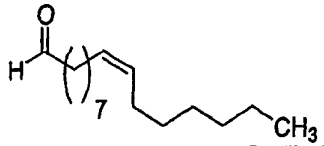
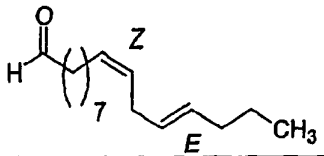
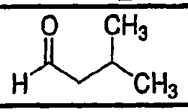
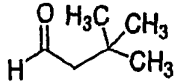
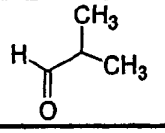
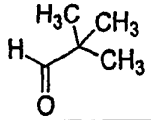
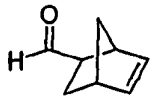
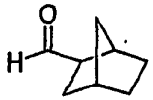
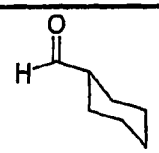
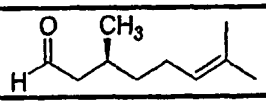
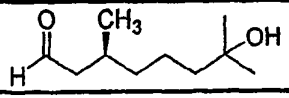
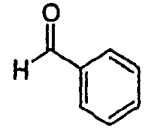
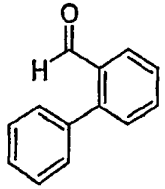
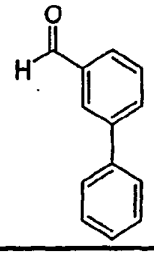
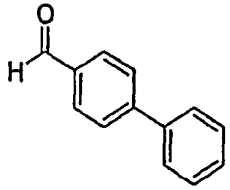
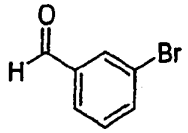
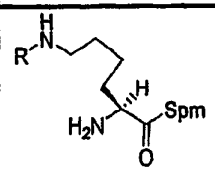
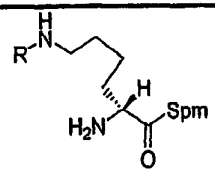
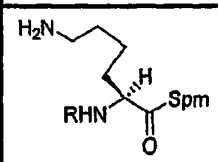
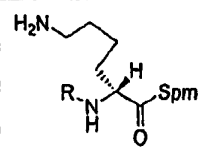
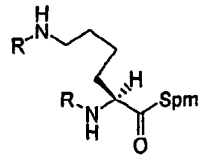
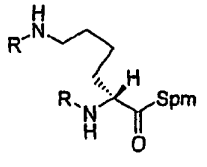
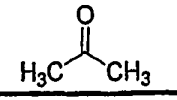
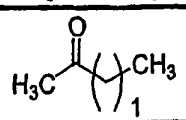
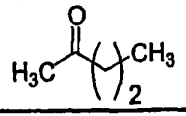
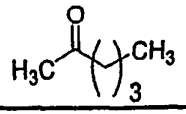
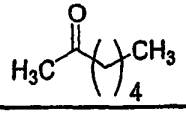
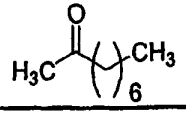
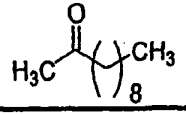
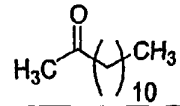
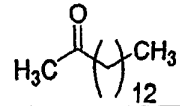
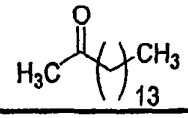
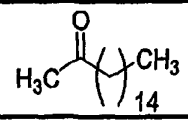
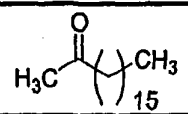
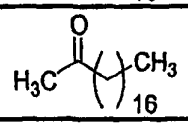
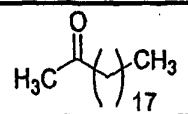
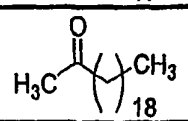
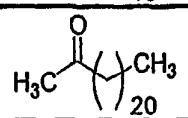
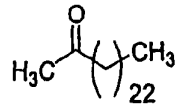
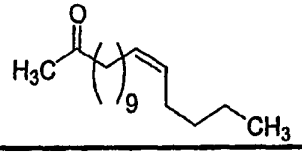
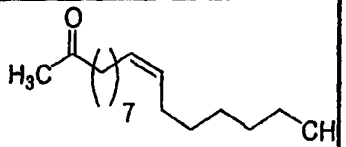
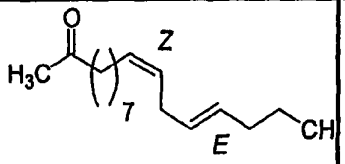
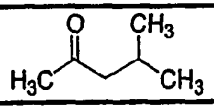
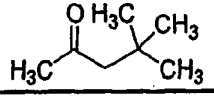
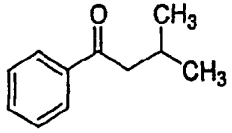
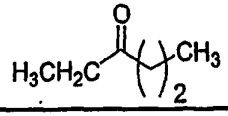
16		IVA16	IVB16	IVC16	IVD16	IVE16	IVF16
17		IVA17	IVB17	IVC17	IVD17	IVE17	IVF17
18		IVA18	IVB18	IVC18	IVD18	IVE18	IVF18
19		IVA19	IVB19	IVC19	IVD19	IVE19	IVF19
20		IVA20	IVB20	IVC20	IVD20	IVE20	IVF20
21		IVA21	IVB21	IVC21	IVD21	IVE21	IVF21
22		IVA22	IVB22	IVC22	IVD22	IVE22	IVF22
23		IVA23	IVB23	IVC23	IVD23	IVE23	IVF23

24		IVA24	IVB24	IVC24	IVD24	IVE24	IVF24
25		IVA25	IVB25	IVC25	IVD25	IVE25	IVF25
26		IVA26	IVB26	IVC26	IVD26	IVE26	IVF26
27		IVA27	IVB27	IVC27	IVD27	IVE27	IVF27
28		IVA28	IVB28	IVC28	IVD28	IVE28	IVF28
29		IVA29	IVB29	IVC29	IVD29	IVE29	IVF29
30		IVA30	IVB30	IVC30	IVD30	IVE30	IVF30
31		IVA31	IVB31	IVC31	IVD31	IVE31	IVF31

32		IVA32	IVB32	IVC32	IVD32	IVE32	IVF32
33		IVA33	IVB33	IVC33	IVD33	IVE33	IVF33
34		IVA34	IVB34	IVC34	IVD34	IVE34	IVF34
35	CH_2O	IVA35	IVB35	IVC35	IVD35	IVE35	IVF35

		A	B	C	D	E	F
	SERIES V	 A (ε-*L*-Lys)	 B (ε-*D*-Lys)	 C (α-*L*-Lys)	 D (α-*L*-Lys)	 E (α,ε-*L*-Lys)	 F (α, ε-*D*-Lys)
1		**VA1**	**VB1**	**VC1**	**VD1**	**VE1**	**VF1**
2		**VA2**	**VB2**	**VC2**	**VD2**	**VE2**	**VF2**
3		**VA3**	**VB3**	**VC3**	**VD3**	**VE3**	**VF3**
4		**VA4**	**VB4**	**VC4**	**VD4**	**VE4**	**VF4**
5		**VA5**	**VB5**	**VC5**	**VD5**	**VE5**	**VF5**
6		**VA6**	**VB6**	**VC6**	**VD6**	**VE6**	**VF6**
7		**VA7**	**VB7**	**VC7**	**VD7**	**VE7**	**VF7**

8		VA8	VB8	VC8	VD8	VE8	VF8
9		VA9	VB9	VC9	VD9	VE9	VF9
10		VA10	VB10	VC10	VD10	VE10	VF10
11		VA11	VB11	VC11	VD11	VE11	VF11
12		VA12	VB12	VC12	VD12	VE12	VF12
13		VA13	VB13	VC13	VD13	VE13	VF13
14		VA14	VB14	VC14	VD14	VE14	VF14
15		VA15	VB15	VC15	VD15	VE15	VF15
16		VA16	VB16	VC16	VD16	VE16	VF16

17		VA17	VB17	VC17	VD17	VE17	VF17
18		VA18	VB18	VC18	VD18	VE18	VF18
19		VA19	VB19	VC19	VD19	VE19	VF19
20		VA20	VB20	VC20	VD20	VE20	VF20
21		VA21	VB21	VC21	VD21	VE21	VF21
22		VA22	VB22	VC22	VD22	VE22	VF22
23		VA23	VB23	VC23	VD23	VE23	VF23
24		VA24	VB24	VC24	VD24	VE24	VF24

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26		VA26	VB26	VC26	VD26	VE26	VF26
27		VA27	VB27	VC27	VD27	VE27	VF27
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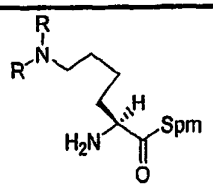
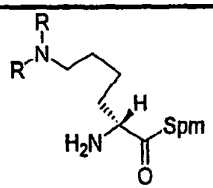
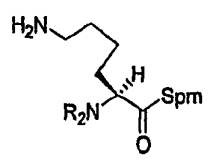
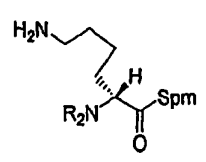
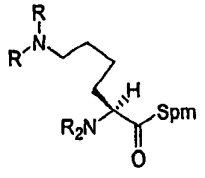
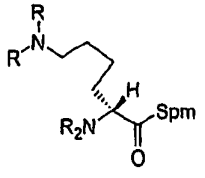
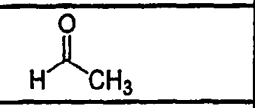
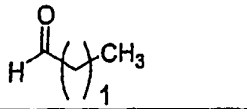
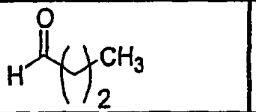
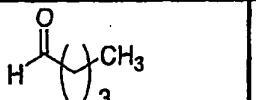
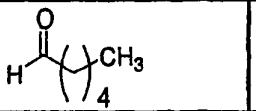
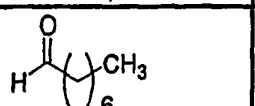
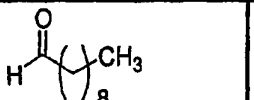
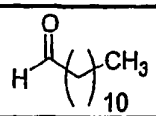
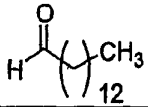
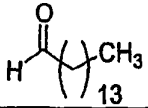
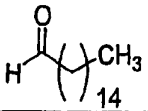
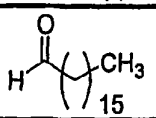
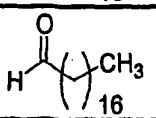
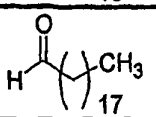
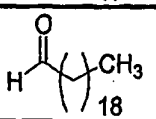
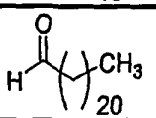
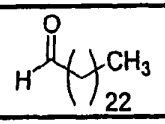
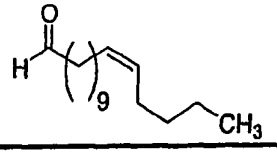
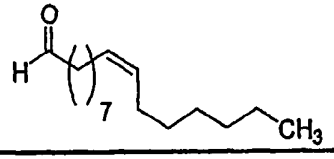
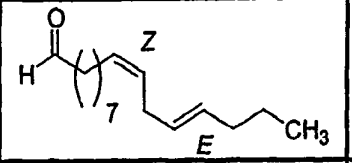
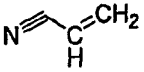
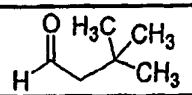
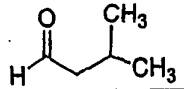
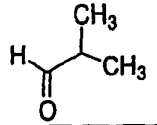
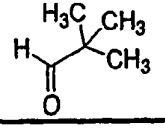
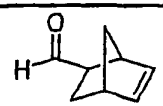
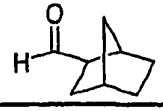
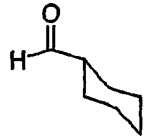
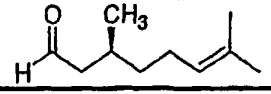
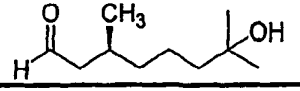
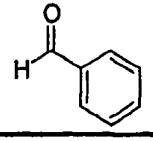
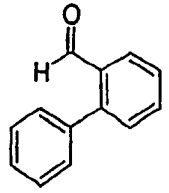
	SERIES VI	 A (ε-*L*-Lys)	 B (ε-*D*-Lys)	 C (α-*L*-Lys)	 D (α-*L*-Lys)	 E (α,ε-*L*-Lys)	 F (α, ε-*D*-Lys)
1		VIA1	VIB1	VIC1	VID1	VIE1	VIF1
2		VIA2	VIB2	VIC2	VID2	VIE2	VIF2
3		VIA3	VIB3	VIC3	VID3	VIE3	VIF3
4		VIA4	VIB4	VIC4	VID4	VIE4	VIF4
5		VIA5	VIB5	VIC5	VID5	VIE5	VIF5
6		VIA6	VIB6	VIC6	VID6	VIE6	VIF6
7		VIA7	VIB7	VIC7	VID7	VIE7	IVIF7

8		VIA8	VIB8	VIC8	VID8	VIE8	VIF8
9		VIA9	VIB9	VIC9	VID9	VIE9	VIF9
10		VIA10	VIB10	VIC10	VID10	VIE10	VIF10
11		VIA11	VIB11	VIC11	VID11	VIE11	VIF11
12		VIA12	VIB12	VIC12	VID12	VIE12	VIF12
13		VIA13	VIB13	VIC13	VID13	VIE13	VIF13
14		VIA14	VIB14	VIC14	VID14	VIE14	VIF14
15		VIA15	VIB15	VIC15	VID15	VIE15	VIF15
16		VIA16	VIB16	VIC16	VID16	VIE16	VIF16

17		VIA17	VIB17	VIC17	VID17	VIE17	VIF17
18		VIA18	VIB18	VIC18	VID18	VIE18	VIF18
19		VIA19	VIB19	VIC19	VID19	VIE19	VIF19
20		VIA20	VIB20	VIC20	VID20	VIE20	VIF20
21		VIA21	VIB21	VIC21	VID21	VIE21	VIF21
22		VIA22	VIB22	VIC22	VID22	VIE22	VIF22
23		VIA23	VIB23	VIC23	VID23	VIE23	VIF23
24		VIA24	VIB24	VIC24	VID24	VIE24	VIF24
25		VIA25	VIB25	VIC25	VID25	VIE25	VIF25

26		VIA26	VIB26	VIC26	VID26	VIE26	VIF26
27		VIA27	VIB27	VIC27	VID27	VIE27	VIF27
28		VIA28	VIB28	VIC28	VID28	VIE28	VIF28
29		VIA29	VIB29	VIC29	VID29	VIE29	VIF29
30		VIA30	VIB30	VIC30	VID30	VIE30	VIF30
31		VIA31	VIB31	VIC31	VID31	VIE31	VIF31
32		VIA32	VIB32	VIC32	VID32	VIE32	VIF32

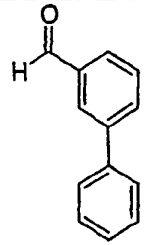
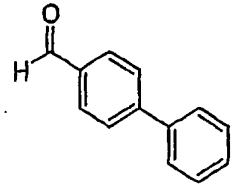
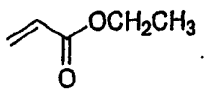
33		VIA33	VIB33	VIC33	VID33	VIE33	VIF33
34		VIA34	VIB34	VIC34	VID34	VIE34	VIF34
35		VIA35	VIB35	VIC35	VID35	VIE35	VIF35
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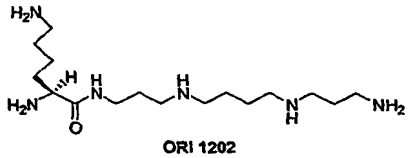
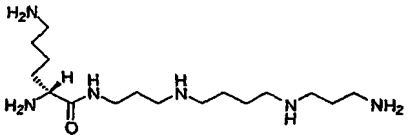
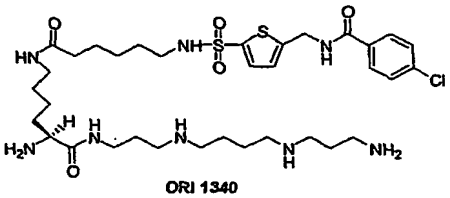
Figure 3



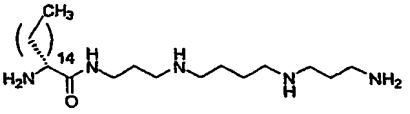
ORI 1202



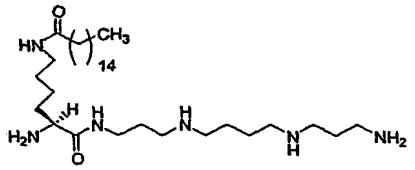
ORI 1426



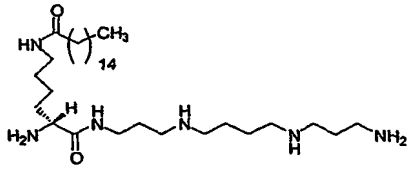
ORI 1340



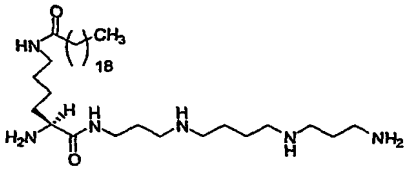
ORI 999



IA4



IB4



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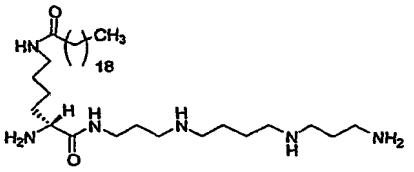
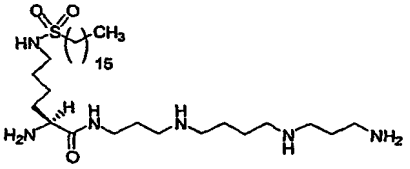
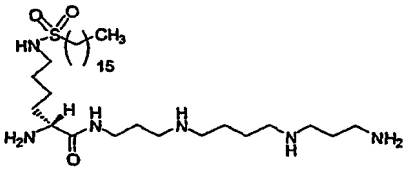


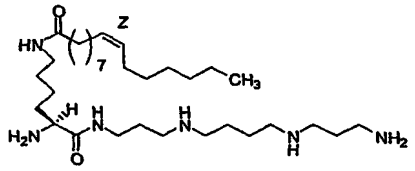
IB1



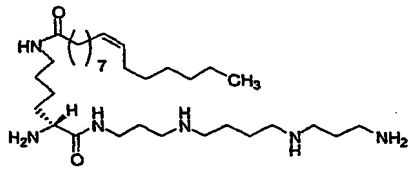
IIA10



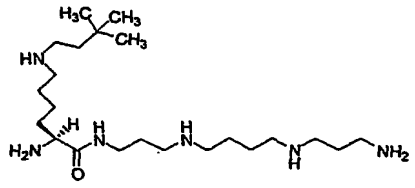
IIB10



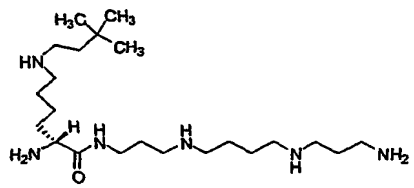
IIIA1



IIIB1



IVA22



IVB22

Figure 4:

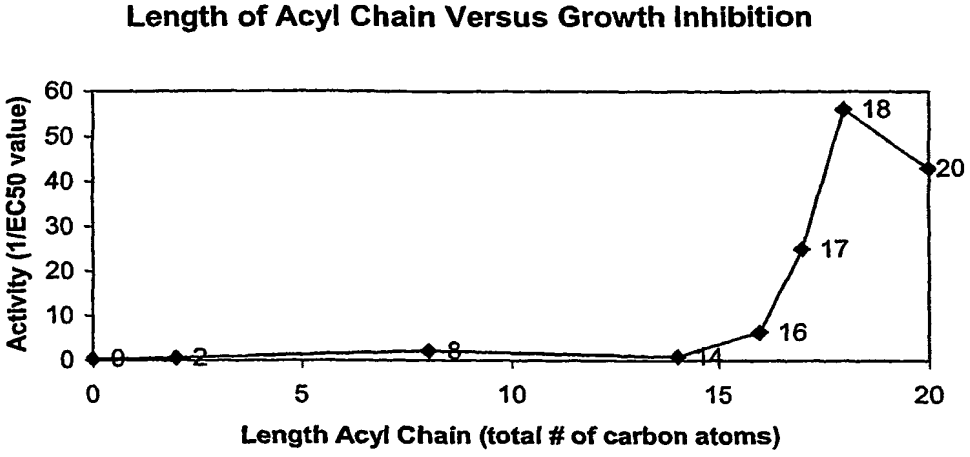
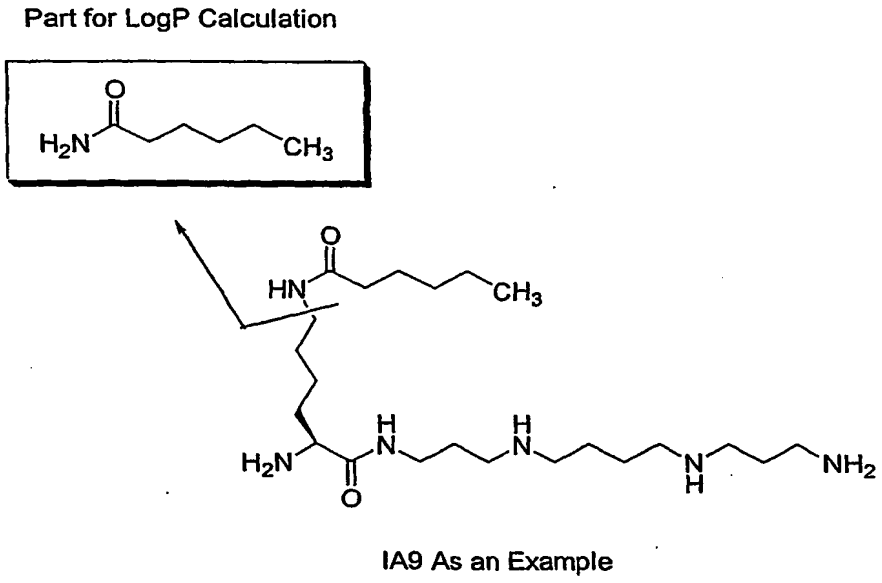


Figure 5:



IA9 As an Example

Figure 6

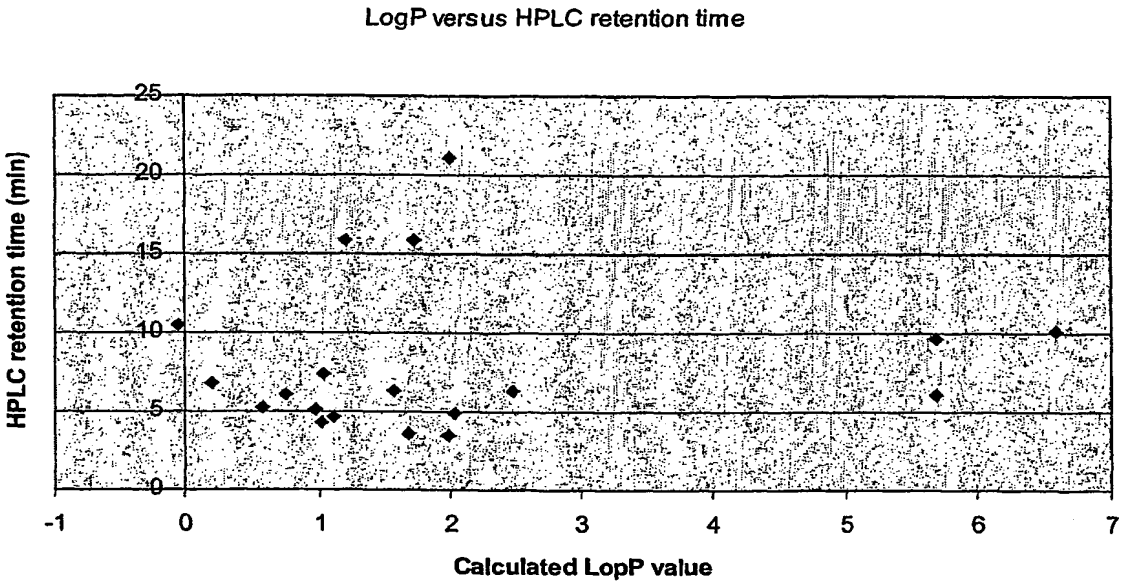


Figure 7

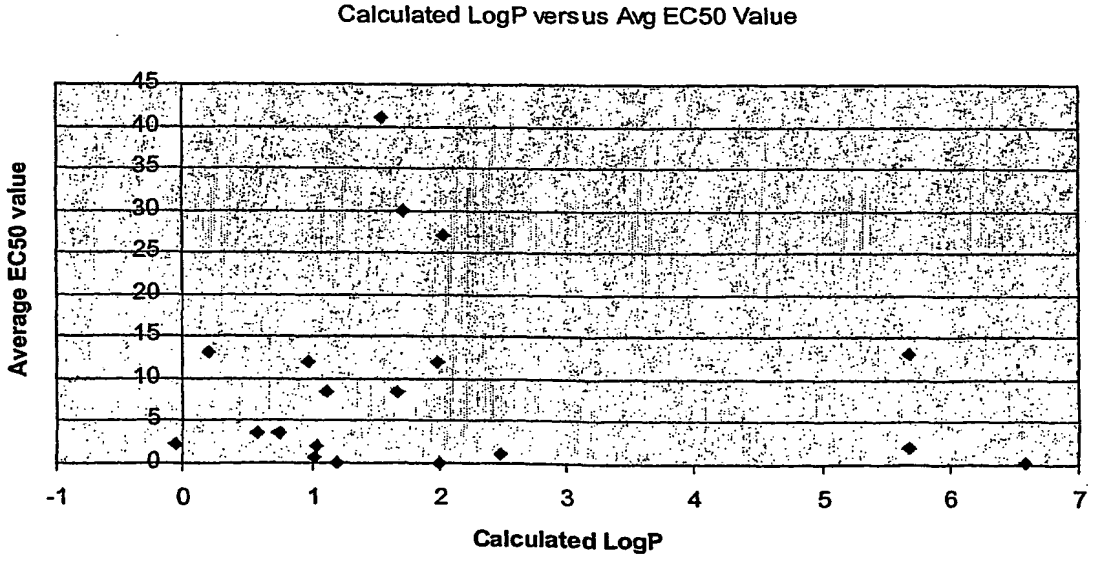
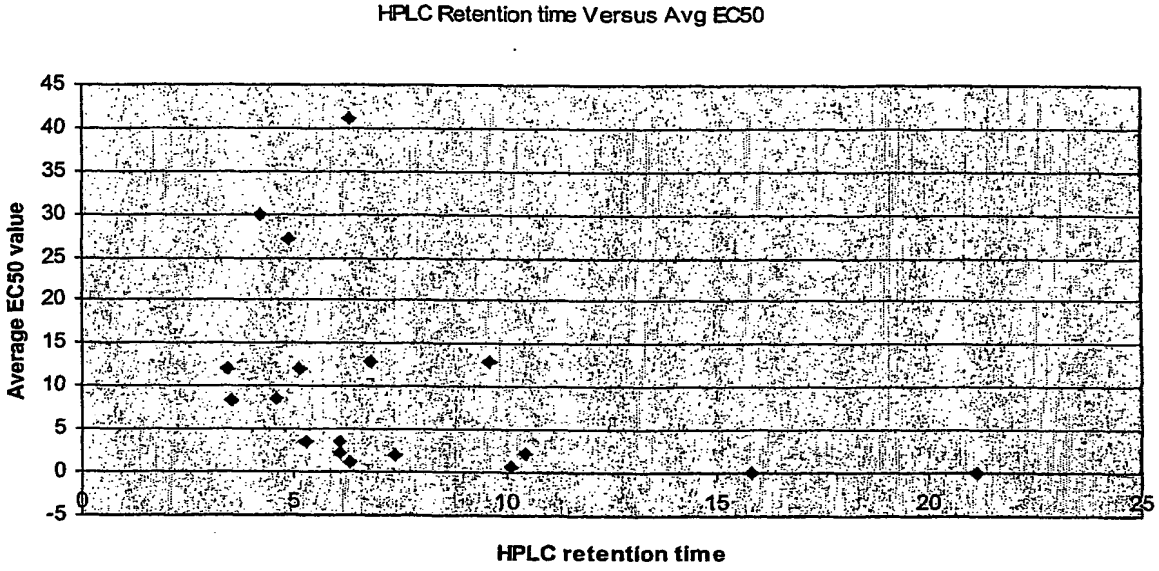


Figure 8



5

Figure 9:

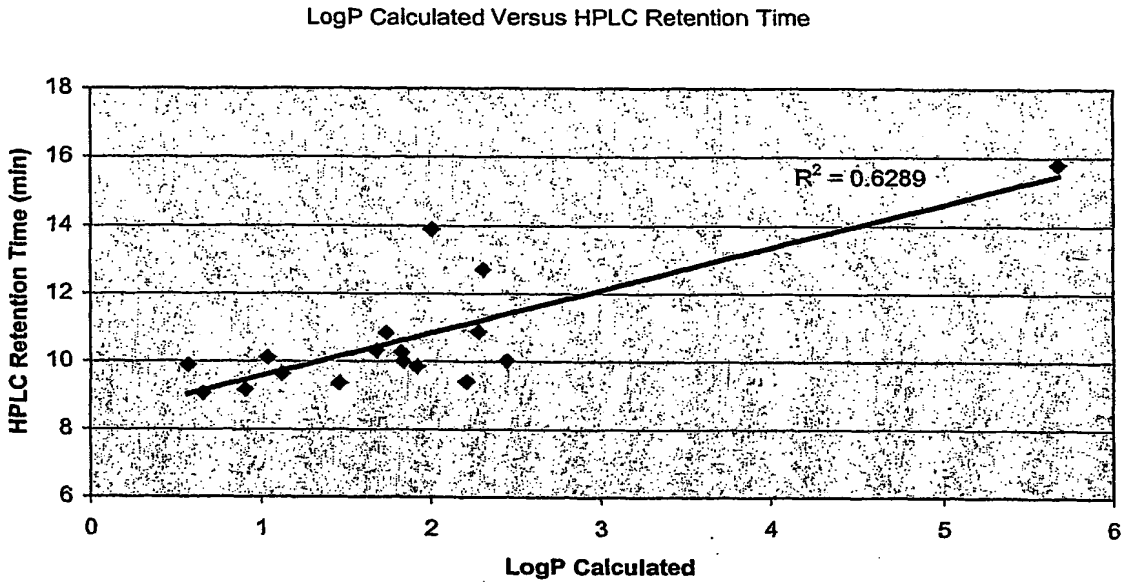


Figure 10:

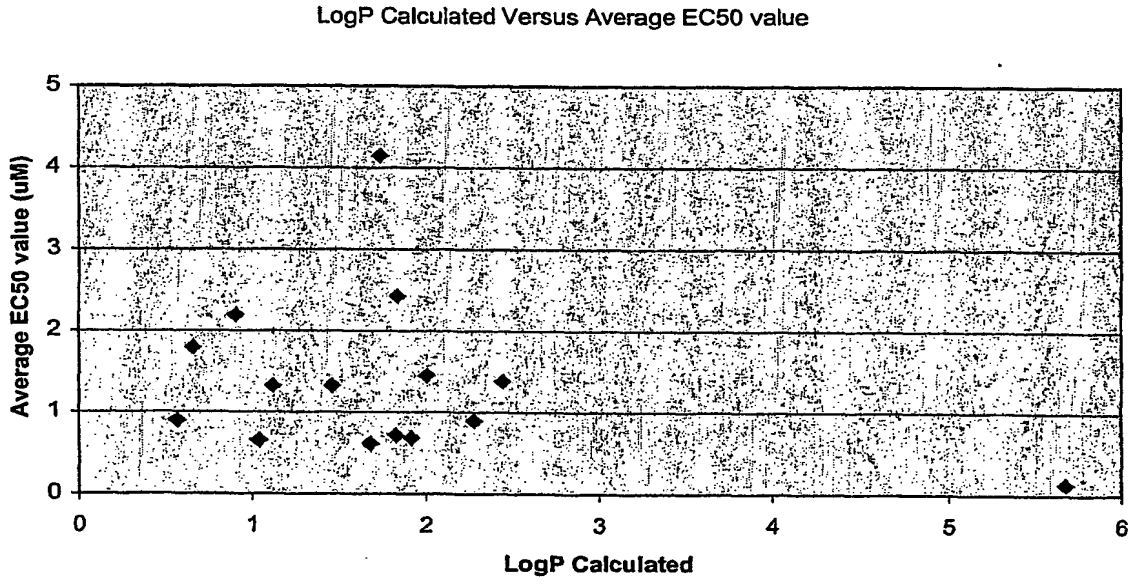


Figure 11:

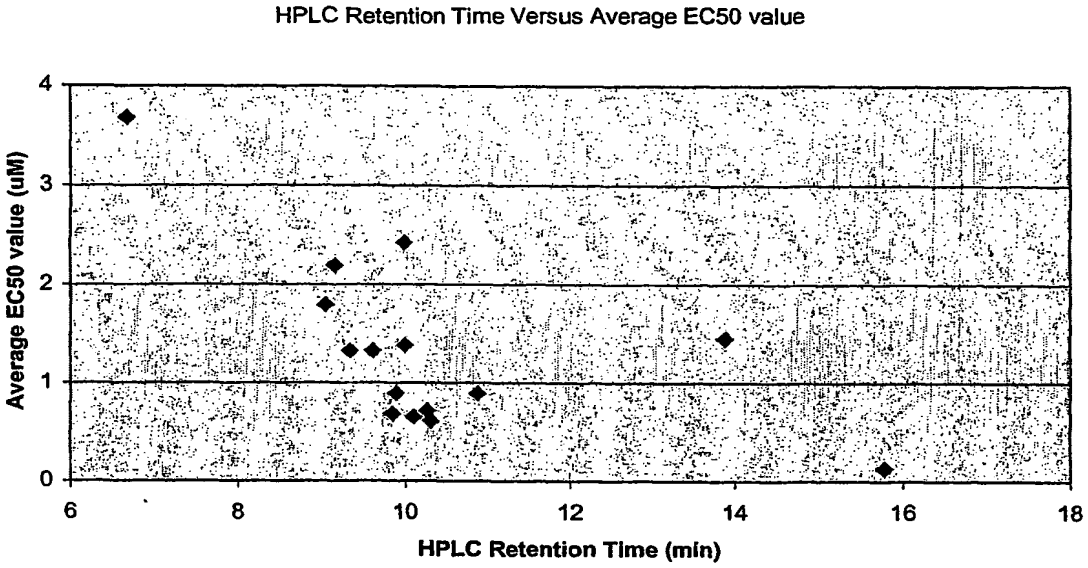

HPLC Retention Time Versus Average EC50 value
Average EC50 value (uM)
0
1
2
3
4
6
8
10
12
14
16
18
HPLC Retention Time (min)

Figure 12

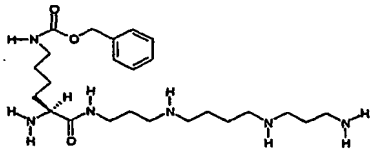
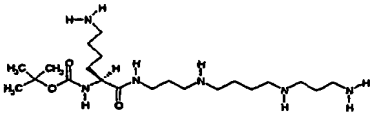
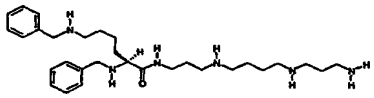
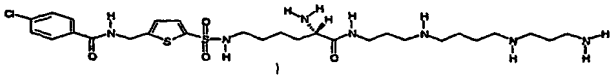
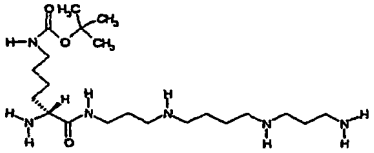
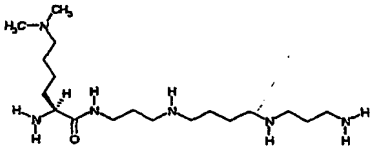
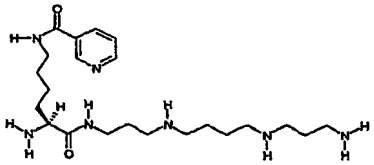
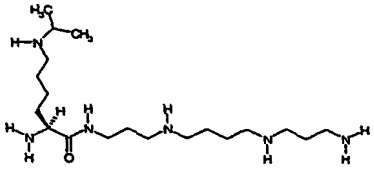
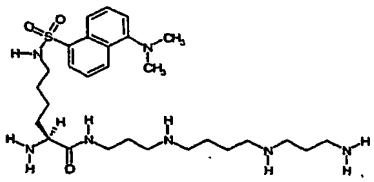
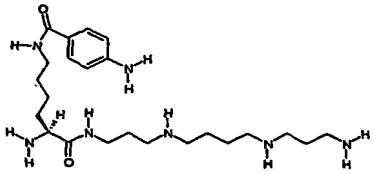
ID	Structure
IA40	
IC41	
IVE30	
IIA21	
IB41	
VIA36	
IA27	
VA1	
IIA20	
IA28	

Figure 12

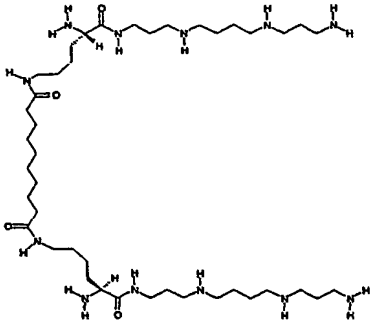
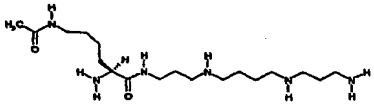
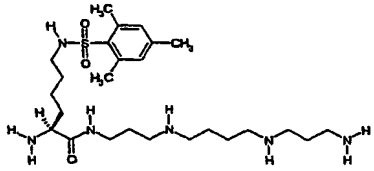
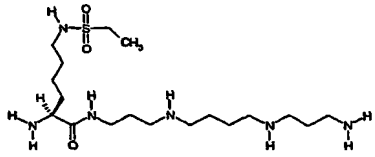
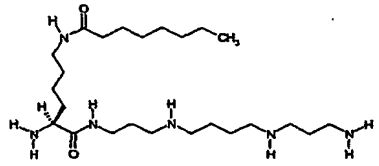
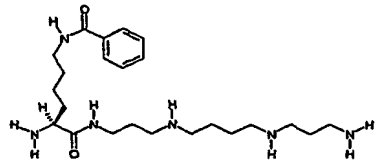
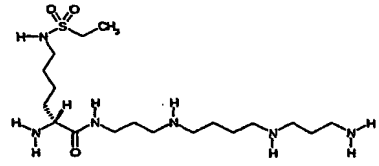
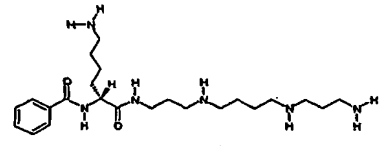
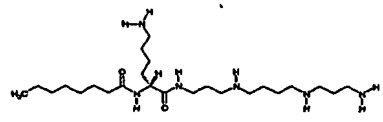
ID	Structure
IA19	
IA11	
IIA17	
IIA2	
IA7	
IA24	
IIB2	
ID24	
ID7	

Figure 12

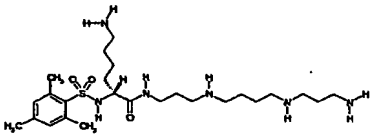
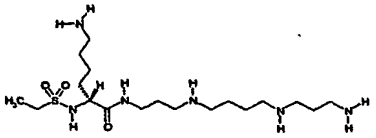
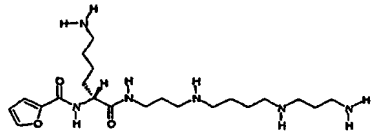
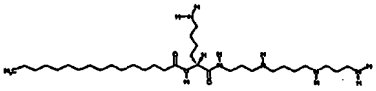
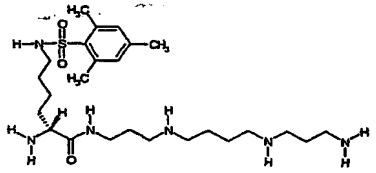
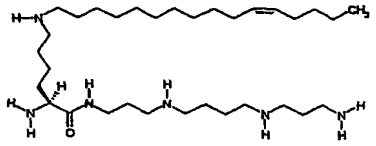
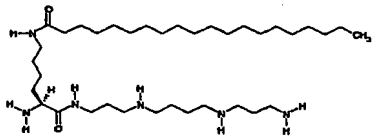
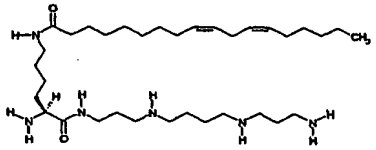
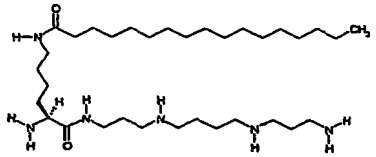
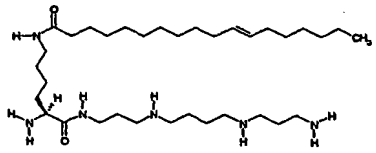
ID	Structure
IID17	
IID2	
ID25	
ID4	
IIB17	
IVA18	
IA1	
IIIA5	
IA3	
IIIA4	

Figure 12

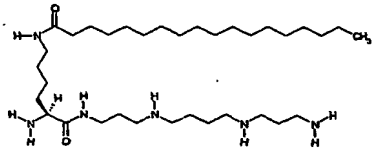
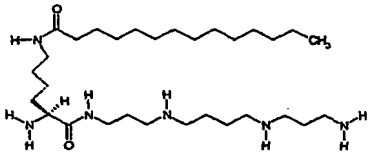
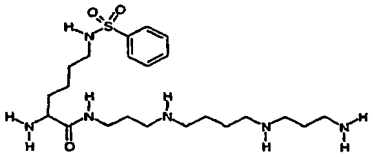
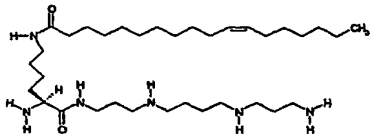
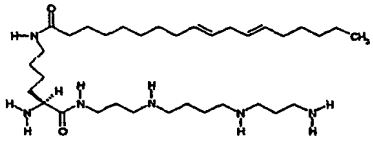
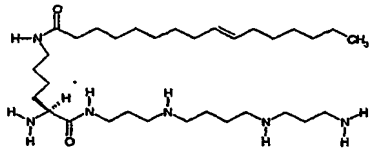
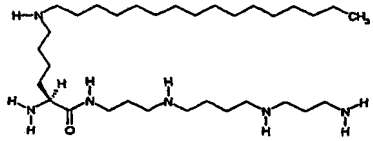
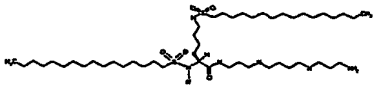
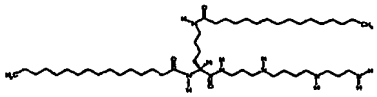
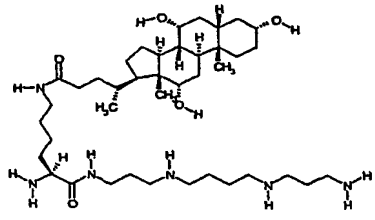
ID	Structure
IA2	
IA5	
IIA16	
IIIA3	
IIIA6	
IIIA2	
IVA11	
IIE10	
IE4	
IIIA7	

Figure 12

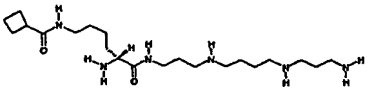
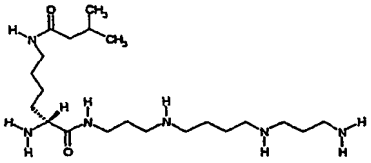
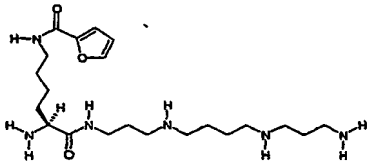
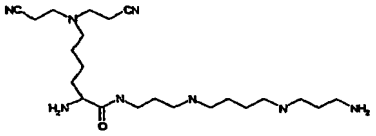
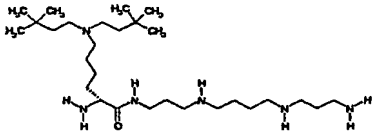
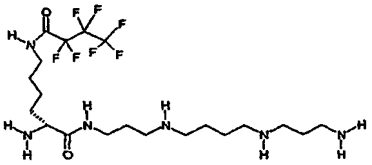
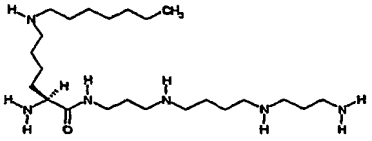
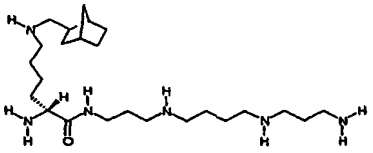
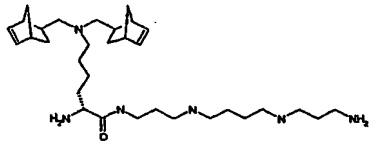
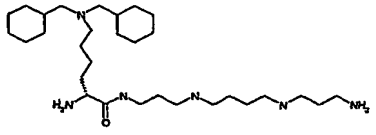
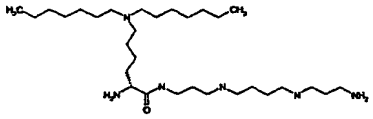
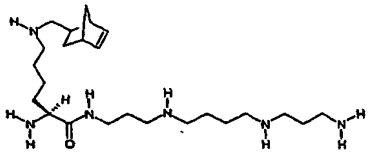
ID	Structure
XXX	
IB35	
IA25	
VIA21	
VIB22	
IB39	
IVA6	
IVB26	
VIB26	
IVF27	

Figure 12

ID	Structure
IVF6	
IVA25	
IVA27